JOURNAL OF AGRICULTURAL RESEARCH

VOL. 47 WASHINGTON, D. C., OCTOBER 1, 1933 NO. 7

SOME FACTORS INFLUENCING THE VARIABILITY IN LENGTH OF COTTON FIBERS ON INDIVIDUAL PLANTS AS SHOWN BY THE SORTER METHOD[1]

By G. M. ARMSTRONG, *botanist and plant pathologist*, and C. C. BENNETT, *research assistant, South Carolina Agricultural Experiment Station*[2]

INTRODUCTION

It is a well-known fact that individual fibers on a seed of cotton differ in lengths. The length of staple as determined by the ordinary method of pulling and combing refers to only one or two of the largest groups of fibers of approximately the same length, and gives no information as to the relative proportions of short fibers or very long fibers. By the use of the Baer and Johannsen mechanical fiber sorters, fibers were separated into groups according to length. Variations between samples were thus revealed. Such methods when used on samples taken from bales of cotton in commerce may yield little information of value to the plant physiologist or plant breeder, but when used on samples obtained from the seed of a variety grown under known conditions, they show some significant differences. A study of such material was undertaken to determine some of the factors which affect lint length.

EXPERIMENTAL METHODS

In 1929, seed of *Gossypium hirsutum* L., variety Super-Seven, strain 4, was obtained directly from the breeder and planted in both greenhouse and field. The field plot, of Cecil clay loam, which usually yields about a bale per acre if given liberal applications of phosphorus, potash, and nitrogen, was divided so that one half received these materials and the other half received none. In the greenhouse, plants were grown directly in the floor soil. They were given liberal applications of commercial fertilizer and watered regularly. The plants were normal, but attained a considerably greater size than those grown in the field. A distinct second crop of mature bolls was produced in the greenhouse but not in the field.

Small groups of plants were selected from various sections of the plots, uniformity in size and spacing being particularly noted. All bolls on the selected plants were self-pollinated. Each boll was tagged on the day of blooming, the branch number and position on the branch being recorded on the tag. The open bolls were picked regularly, usually twice a week, and the date of picking noted.

Seed produced from selfed flowers of 1929 was available for planting in 1930, and all flowers on selected plants were again self-pollinated as during the preceding season. In the field and the greenhouse the same plots were used as in 1929, and the general procedure was the same.

[1] Received for publication Sept. 9, 1932; issued October, 1933. Technical Contribution No. 26 (new series) from the South Carolina Agricultural Experiment Station.

[2] The authors are indebted to Director H. W. Barre, coleader of the project, for the helpful suggestions given during the progress of this work.

A suitable room for arraying the fibers was available through the courtesy of the textile department of Clemson College. The humidity of the room was kept at 70 percent by automatically controlled humidifiers. The normal variation was within a range of 1 percent. Both Johannsen and Baer sorters were used by the junior author, who made the arrays. The fibers were weighed on a Zweigle balance which was checked against two analytical balances of precision. To insure a representative sample for the sorter, the fibers were carefully pulled by hand from every seed in each boll and thoroughly mixed in the manner described by Balls (*5, p. 59*).[3]

The methods of measuring the daily growth of fibers, the volume of seed, the volume of boll, and the number of fibers per seed are described later in connection with the discussion of these operations.

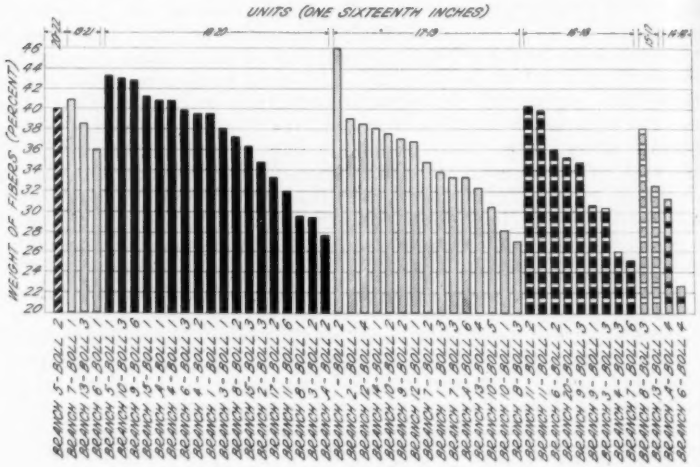

FIGURE 1.—Percentage of the total weight of the cotton fibers occurring in the three largest adjacent length groups of the sorter array for each boll of greenhouse plant no. 13. Adjacent length groups of the array differ by one sixteenth of an inch.

VARIABILITY OF THE LINT ON AN INDIVIDUAL PLANT

To determine the variability in lint length within a single plant, fiber arrays were made of the lint of each boll from plants grown in the greenhouse and in the field.

The data for plant no. 13, grown in the greenhouse in 1929, are presented in part in figure 1, as fairly representative of all the plants, though no plant in the field produced so many bolls. There was a tendency for bolls formed late in the season on the plants in the field to produce shorter lint, probably because of dry weather. A detailed presentation of the data for plant 13 only would require 50 large tables; thus, much of it is necessarily omitted.

In figure 1 the boll number refers to the node occupied by the boll along the branch from the stem outward. Vegetative branches are indicated from the base upward by letters, *A* and *B*, and fruiting branches

[3] Reference is made by number (italic) to Literature Cited, p. 465.

by numbers. For the sake of brevity certain symbols are used in the discussion which follows; for example, 6-4 indicates the boll at the fourth node on fruiting branch no. 6. To translate the sorter distribution into the approximate terms of the cotton classer, the modal length has been employed. The percentages of the total weight of the three heaviest groups which are adjacent to each other have been added. For example, the boll 6-4 had 7.14 percent of the total lint weight fourteen sixteenths of an inch in length, 8.27 percent fifteen sixteenths of an inch, and 7.22 percent 1 inch. This gives 22.63 percent, as shown in figure 1. The cotton classer would probably call this a cotton with fibers fifteen sixteenths of an inch long, since the lint length of the classer is usually about the 75 percent point on the cumulative weights scale of the sorter array. The term "modal groups", used for the presentation of certain features of the length characteristics of the fibers on a plant, does not adequately describe their chief characteristics, but neither does the mean nor the practical length obtained by the hand-stapling method. Clegg (*11*) shows very clearly that no one expression of results is entirely satisfactory and that a combined stapling test is necessary to bring out such important features of a cotton sample as practical length, percentage dispersion as a measure of uniformity, and percentage of short fibers. A combination of the three heaviest length groups does not give a real measure of the uniformity of fiber length but gives some indications of it. A majority of such modal groups are also the same as the practical length of the hand-stapling test.

In figure 1 the fiber of boll no. 4 on branch no. 6 is the least desirable from the standpoint of length and uniformity. Perhaps the greatest contrast to boll 6-4 in modal groups is 1-2, with 46 percent of the lint in the three heaviest adjacent groups. The fibers of this boll would probably be classed as eighteen or nineteen sixteenths of an inch in length. The boll containing the longest fibers is 5-2, the fibers of which would be classed as twenty-one sixteenths of an inch. The relation of boll position to fiber length and uniformity is discussed in succeeding paragraphs, but it is desired to point out here the six sixteenths of an inch difference in fiber length of two bolls 6-4 and 5-2 located rather close together on the plant. This is a greater difference in fiber length than that usually supposed to exist between varieties bred for variations in length of staple.

A variation of five sixteenths of an inch in fiber length from boll to boll was observed in several of the plants grown in the field, though none of those selected for study produced more than 10 mature bolls.

Humbert and Mogford (*18*) used the combing method to study the lint length of every seed in each lock of an inbred plant of Mebane cotton and also of a few seeds from its progeny. They found that the length of lint varied in different bolls on the same plant, in the same boll, and on seed which were side by side in the same lock. The results here presented show the same essential variation in regard to different bolls. The lint produced in bolls near the top of the Mebane plant had a mean length that did not differ greatly from the length of lint in bolls from other parts of the plant. The results presented in figure 1, for a plant grown in the greenhouse, are in essential agreement with this finding, since boll 13-3 ranks third, 10-3 ranks sixth, and 15-1 ranks eighth. Bolls at the top of plants growing in the field, however, showed a tendency to produce shorter lint. Ewing (*13*)

and Venkataramanan (*25*) also found shortening of lint in upper bolls, probably due to increasing drought, but Kearney and Harrison (*20*) found that bolls borne on the lower fruiting branches of the Pima variety of Egyptian cotton produced shorter fibers than bolls higher on the plant.

Not only were there decided differences in the length of the modal groups, but there were also appreciable differences in the uniformity of distribution of the fibers of various lengths, as is seen in figure 2. The curve for boll 1-2 shows a greater tendency to flatten, and the boll therefore possesses a rather large percentage of fibers in a few length groups, 46 percent falling in the groups seventeen to nineteen sixteenths of an inch. Boll 6-4 has only 22.63 percent of its fibers in any three adjoining length groups, whereas boll 5-2 is intermediate in this respect with 40 percent of the fibers in three groups. If a

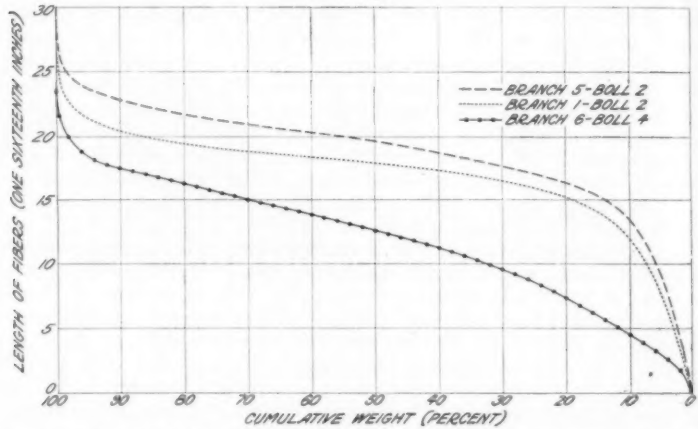

FIGURE 2.—Variation in the distribution of the length groups of sorter arrays of the lint from three bolls of greenhouse plant no. 13.

uniform staple of a particular length is really desired, then boll 1-2 is perhaps the best on this plant, though there are on the plant 14 other bolls that the cotton classer would probably grade as the same length and 22 others that have longer staple lengths.

A study of lint length finally resolves itself into a study of cell nutrition, which is complicated by numerous variable factors. Perhaps the single seed would be the best unit for study of the competition between seed and lint for the available nutrients, but for practical reasons the boll has been used as the unit in this study. The following points were considered in their relation to lint length on individual plants: The effect of (1) position of seed in a single boll, (2) position of bolls of the same age on a single plant, and (3) age as well as position of bolls on a single plant.

EFFECT OF POSITION OF SEED IN A SINGLE BOLL

Only a few arrays were made of the fibers of every seed in a single lock. As an example of the variation to be found, the results from a lock of boll no. 1 on the sixth fruiting branch of plant no. 6, grown in

the greenhouse, are given in table 1. If the percentage weights of the three heaviest adjoining length groups are used as the probable staple length, it will be seen that seeds nos. 1 and 2 at the base of the lock have the shortest lint length, which is seventeen sixteenths of an inch. Seeds 3, 4, and 8 have fibers nineteen sixteenths of an inch in length; seeds 5, 7, 9, and 10, twenty sixteenths of an inch; and seed 6, twenty-one sixteenths of an inch. Thus, there is a variation of five sixteenths of an inch in lint length of the seed from a single lock.

TABLE 1.—*Sorter distribution of fiber lengths on each seed from a l*

[Seed numbered from the base of locule upward]

Lengths in sixteenths of an inch	Percentage of the total fiber weight in each length group for seed no.—									
	1	2	3	4	5	6	7	8	9	10
29							0.02			
28									0.02	
27			0.09			0.14	.09	0.04		
26	0.09		.35	0.10	0.10	.23	.19	.09	.17	0.06
25	.09	0.16	1.13	.86	.77	1.05	1.49	.36	.27	.20
24	1.50	.24	2.78	1.44	2.68	3.67	3.45	1.26	1.65	.80
23	5.02	.47	4.70	4.12	5.04	6.38	6.16	2.07	4.55	4.82
22	5.28	1.98	4.00	5.94	8.43	11.71	8.96	4.68	8.27	9.23
21	8.80	3.88	8.96	9.38	9.29	* **12.15**	17.64	10.08	15.51	14.65
20	10.48	6.33	12.96	12.83	* **13.70**	16.69	* **13.85**	17.28	* **15.51**	* **13.85**
19	12.15	10.93	* **14.61**	* **12.83**	13.70	8.91	13.63	* **22.94**	12.41	10.64
18	12.41	13.85	10.26	10.44	6.90	6.38	12.14	11.52	11.99	10.54
17	* **13.82**	* **14.73**	10.61	8.10	7.85	4.54	5.00	6.48	8.89	9.23
16	13.12	12.51	6.09	5.75	5.56	4.28	4.48	5.22	3.72	6.42
15	4.75	7.76	4.52	6.90	4.98	4.37	2.05	2.79	2.79	3.81
14	3.26	6.02	3.13	2.87	3.54	4.20	1.31	1.98	3.10	2.61
13	1.94	5.86	1.91	1.92	3.06	1.57	1.12	2.43	1.65	2.01
12	.62	3.40	1.91	1.21	1.72	1.75	.56	1.44	.83	1.10
11	1.06	2.06	1.57	1.44	.57	1.57	.19	1.17	.83	.50
10	.79	1.82	1.39	1.44	1.19	1.31	.28	.81	.83	.50
9	.79	.95	1.22	1.05	.96	.87	.56	1.08	.72	.90
8	.53	1.11	.87	1.72	1.72	.87	.75	.72	.52	.50
7	.26	.95	.70	1.24	1.34	1.22	.56	.36	.52	.80
6	.44	.63	1.04	1.72	1.15	1.40	1.08	.72	.89	.50
5	.44	.55	.87	1.15	1.15	1.05	.75	.81	.41	1.20
4	.44	.95	1.22	1.53	1.44	1.14	.65	1.08	1.24	1.20
3	.97	1.74	.87	1.53	1.34	.96	.93	.81	1.03	1.40
2	.97	1.11	2.26	2.49	1.82	1.57	1.49	1.80	1.65	2.51

* Boldface figures show modal length and emphasize increase in length of lint from seed 1 to 6 and then a slight decline.

If a curve were drawn through the boldface lint lengths in table 1, it would serve to emphasize the increase in length of lint from seed 1 to seed 6, inclusive, and then the slight decline in seeds 7, 8, 9, and 10. Henry (*17*) notes the same general trend of lint length from base to tip of a lock in Egyptian and sea-island cottons. Humbert and Mogford (*18*) call attention to the variation in length of lint from seeds located side by side in a lock. Ayyar and Rao (*1*) state that in a lock of *Gossypium hirsutum* grown in India the first seed produced the shorter lint, and there was no difference in length of lint among the other seeds in a lock.

The distribution of the various lengths is also quite variable, as table 1 shows. If we assume that a sample desired for spinning is one with the greatest percentage of the weight in closely adjoining length groups, and arbitrarily take those groups with 10 percent or more in each for comparison, the fiber from seed 1, with 61.98 percent of the weight in 5 length groups, is the best, and that from seed 5, with 27.4 percent in 2 length groups, is the poorest. Seed 8, with 61.82 percent of the fibers in 4 length groups, is a close second to seed 1, according to the assumed standard of uniformity.

A comparison of the seeds on the basis of the percentage of fibers less than eleven sixteenths of an inch in length also places seed 1 first, with 5.63 percent of the fibers in this group, while seed 4, with 13.87 percent in this group, would probably have the greatest waste of short fibers in the spinning process. Seed 6, with the longest fibers, has 10.39 percent of the above-mentioned short fibers.

EFFECT OF POSITION OF BOLLS OF THE SAME AGE ON A SINGLE PLANT

Because of the impossibility of obtaining many bolls from flowers of 1 day on a single plant, a comparison was made of several bolls from flowers on 2 successive days, namely, July 8 and 9 and July 21 and 22. Bolls 2-3, 3-3, 5-2, 6-2, and 8-1 developed from blooms on the first-mentioned dates, and curves of the arrays are shown in figure 3. There is a difference of four sixteenths of an inch in length of lint of modal groups between bolls 3-3 and 5-2 and also a very appreciable

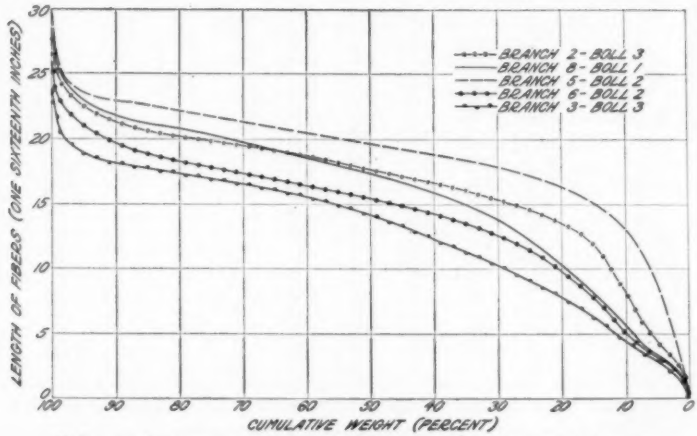

FIGURE 3.—Sorter arrays of lint from bolls flowering July 8 and 9 on plant no. 13.

difference in the distribution of the different lint lengths, boll 3-3 being inferior to boll 5-2.

Four bolls, 8-3, 9-3, 11-2, and 13-1, developed from blooms on July 22, and one, 6-4, from a bloom on July 21. In the first-mentioned bolls there was considerable uniformity in the distribution of the various lint lengths (fig. 4), but in boll 6-4 there were decidedly more short fibers. These examples, as well as the results from other plants, which will not be presented, show that even though the bolls may develop under about the same external environmental conditions, there is much variation in the distribution of the fibers of various lengths and in the final length attained.

EFFECT OF AGE AS WELL AS POSITION OF THE BOLLS ON A SINGLE PLANT

POSITION ALONG THE MAIN AXIS

The curves from the arrays of fibers for all bolls that matured on the first node of each fruiting branch from nos. 1 to 20, inclusive, are shown in figure 5. Bolls 8-1 and 10-1 are outstanding because of the

percentage of short fibers that they contain, although in figure 1 they are classed as nineteen sixteenths and eighteen sixteenths of an inch in length, respectively. It appears from table 2 that conditions for lengthening of lint in bolls at the first node were favorable for those on branches 1 to 7, inclusive, the longest lint (twenty sixteenths of an inch) occurring in bolls 6-1 and 7-1. Except for the slight variation in bolls 9-1 and 11-1, conditions then appeared to become progressively less favorable, since lint length for boll 8-1 was nineteen sixteenths of an inch, for 10-1 and 12-1 eighteen sixteenths of an inch, and for 13-1 only 1 inch. Conditions apparently again became more favorable, as lint length was eighteen and nineteen sixteenths of an inch for bolls 14-1 and 15-1. Unfavorable conditions for bolls at the first node followed, as no bolls were retained at first nodes on branches 16, 17, 18, and 19, though a boll at the first node on branch 20 produced relatively short lint, seventeen sixteenths of an inch in length. Even

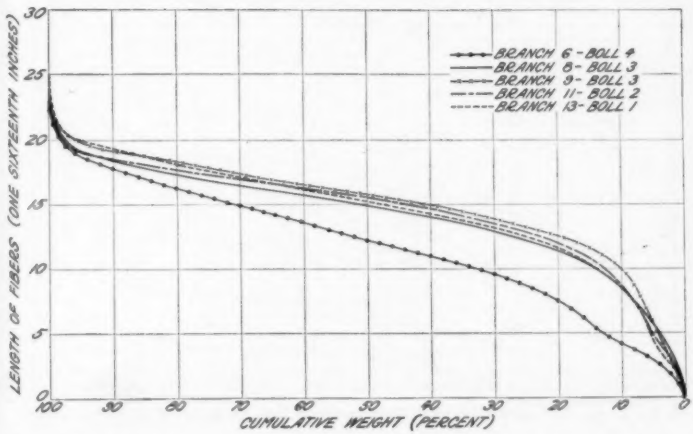

FIGURE 4.—Sorter arrays of lint from bolls flowering July 21 and 22 on plant no. 13.

though it has been previously shown that there are differences in lint characteristics of bolls from flowers of the same day or for periods of 2 or 3 days, indicating that specific nutritional conditions may be localized to some extent, such progressive changes as the foregoing seem to indicate that gradual changes in those factors that influence the nutrition of seed and lint are also operative. Two factors of indicated importance are water supply (*3*, *4*, *21*, *23*) and boll load (*12*), though much remains to be elucidated in this regard.

In spite of the very decided variation from boll to boll, evidence of some generalized effect is also strengthened if other bolls than those of first nodes are considered. It will be seen in table 2 that bolls from blooms of June 19 to July 8, inclusive, produced relatively long fibers in the modal groups, with a tendency for longest fibers to be produced in bolls from flowers that opened near the latter date. Quite unfavorable conditions apparently occurred from bolls beginning development July 9, 10, and 11, since lint length was two to four sixteenths of an inch shorter than in bolls beginning development July 8 on comparable positions of adjacent limbs.

TABLE 2.—*Date of blooming, position of boll on the plant, and modal lint length for each cotton boll on fruiting branches of plant no. 13, 1929*

Date of blooming	Bolls produced on—				Modal lint length [a] [b] in sixteenths of an inch			
	First nodes	Second nodes	Third nodes	Fourth, fifth, and sixth nodes	First nodes	Second nodes	Third nodes	Fourth, fifth, and sixth nodes
June 19	1-1				19			
June 23	2-1				(19) 18			
June 26	3-1				19			
June 29		1-2				(19) 18		
June 30	4-1				19			
July 2	5-1				(20) 19			
July 3		3-2				19		
July 4	6-1				20			
July 5		4-2	1-3			(20) 19	18	
July 6	7-1				20			
July 8		5-2	2-3			21	19	
July 9	8-1	6-2	3-3		**19**	**17**	**(16) 17**	
July 10	9-1				**(18) 17**			
July 11			4-3				**17**	
July 12		7-2				(19) 18		
July 14	10-1				18			
July 15		8-2	6-3			19	(20) 19	
July 16	11-1	9-2			(19) 17	18		
July 17			7-3				18	
July 18	12-1				18			
July 20		10-2				18		
July 21				6-4				**15**
July 22			8-3				**16**	
July 22	13-1	11-2	9-3		**(17) 16**	**17**	**17**	
July 24	14-1				18			
July 27			10-3				(20) 19	
July 28	15-1				19			
Aug. 3			13-3				20	
Aug. 4								
Aug. 5				7-6				19
Aug. 5				12-4				18
Aug. 8		17-2		13-4		19		(19) 18
Aug. 9			15-3	10-5			19	18
Aug. 9				9-6				19
Aug. 10	20-1				(18) 17			

[a] The cotton classer's staple length is usually about the 75 percent point on the cumulative weights scale of the sorter array. The figures in parentheses give the length nearest the 75 percent point when these do not coincide with the modal grouping.

[b] Boldface figures show shortest fibers which were produced from blooms at 2 short intervals of time.

Lint of greater length was produced in all bolls from succeeding blooms until those appearing July 21 and 22, which produced the shortest lint of all, namely fifteen, sixteen, and seventeen sixteenths of an inch. Later bolls produced longer lint except 20-1, the last one of the first crop to be retained, which produced lint of seventeen sixteenths of an inch. These seem to be rather striking variations in lint lengths in bolls from flowers of successive days. Balls (4) also mentioned, "* * * the extreme rapidity with which the properties of the cotton may change, so that the picking of 1 day may be capable of differentiation from those of the preceding and following days."

POSITION OF BOLL ON THE BRANCH

In addition to the position of the boll along the main axis, which has been considered, its position on the branch may also influence lint length and uniformity.

An examination of table 2 shows that fruiting branches 1, 3, 4, 6, 7, 8, 9, and 10 produced bolls at the first three nodes and branch 6 also produced a boll at the fourth node. There are variations of three

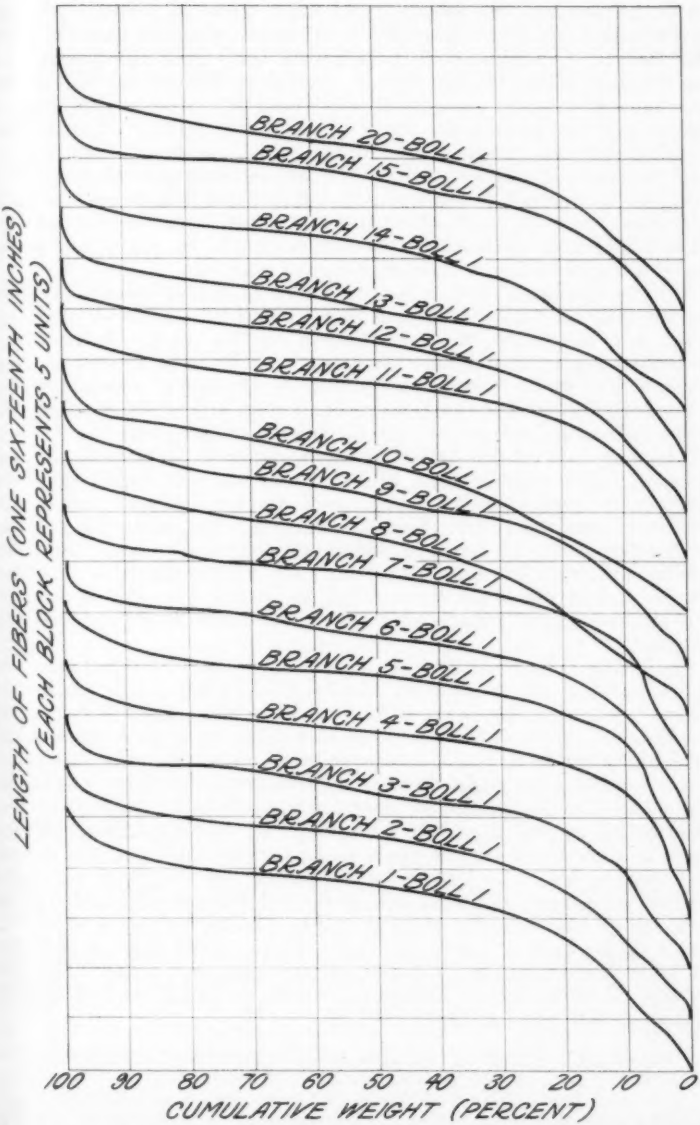

FIGURE 5.—Sorter arrays of lint from all bolls maturing at the first node on each fruiting branch of plan no. 13. Each block represents 5 units.

sixteenths of an inch in the lint length of modal groups from bolls at 3 nodes on 1 limb and of five sixteenths of an inch between the 4 bolls on branch no. 6. The inferiority of boll 6-4 should perhaps be given further consideration. Branch 6 is in an intermediate position along the main axis, which may be considered a fairly favorable position for the transfer of water and organic materials, though the boll at the fourth node may be considered in a relatively unfavorable position because of the three bolls ahead of it on the branch. Three other fruiting branches, however, nos. 7, 9, and 10, produced four bolls on each branch. If the bolls are given a rank according to their position in figure 1, from left to right, the fourth retained boll on each of these branches—namely, 7-6, 9-6, and 10-5—ranks 46, 6,and 35, respectively. This would indicate that the position along the branch is not necessarily a major factor in the inferiority of boll 6-4.

Boll 6-4 developed from a bloom of July 21, and it has already been shown that all bolls developing Juy 21 and 22 produced relatively short lint, regardless of their position on the plant. Some factor or factors acting throughout the plant on bolls from flowers of these dates as well as on those produced June 9, 10, and 11 apparently had a greater effect on the shortening of lint than any of the more specific and localized influences that are considered in this paper.

The lint from all the bolls from five plants in the greenhouse was arrayed. Two other plants, 19 and 20, in addition to 13, show the alternating production of longer and shorter lint, but not so distinctly as does the latter. Such a condition is not evident in the two remaining plants of the five which were studied. Bolls from distinctly different flowering dates on each of the greenhouse plants 13, 19, and 20 show the shorter lint, indicating that it was not general environmental conditions in the greenhouse that produced this condition.

Studies of bolls from the field also show no specific relation of lint characteristics to position on the branch. Fifty limbs from field plants with three or more bolls per limb were selected for study in 1929, and arrays of the fiber from all bolls on 14 of them were made. Fruiting branches 4, 5, and 6 were selected chiefly in order that bolls of comparable ages might be had. Differences in lint length of modal groups or the percentage of fiber ten sixteenths of an inch or less from boll 1 to boll 2 or boll 2 to boll 3 are not significant, though there is a tendency for the fibers to be longer in bolls at the second and third nodes. The figures for the percentage weight of the modal groups seventeen, eighteen, nineteen, and twenty sixteenths of an inch are as follows: For bolls at the first node, 12.79, 14.26, 12.72, and 9.51; for bolls at the second node, 11.25, 12.58, 13.67, and 10.38; for bolls at the third node, 10.76, 11.89, 13.03, and 11.09. The percentage of fibers ten sixteenths of an inch or less at node 1 is 9.47; at node 2, 9.67; and at node 3, 10.39.

PROBABLE TIME OF ORIGIN OF FIBERS AS RELATED TO VARIATIONS IN LINT LENGTH

The majority of the sorter graphs made of lint from the variety of cotton used in these studies approximate the one shown in figure 8 for fibers from 50-day-old bolls. A knowledge of some of the factors which produce this usual type of distribution is necessary before the variations from the type can be at least partly understood.

Balls (*3*) and Hawkins and Serviss (*16*) have stated that the cotton fibers elongate during the first 25 days of boll development, and that wall thickening takes place during the following 25 days. Balls (*3*, *4*) maintains that the individual cells of the epidermis of the seed all sprout at the same stage of boll development, on or near the day when the flower opens. If this is accepted as true, then a

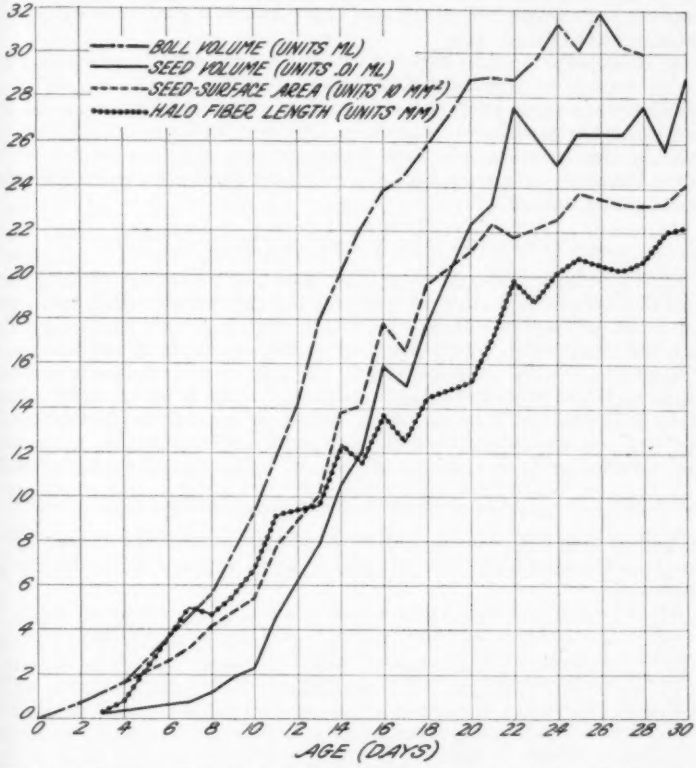

FIGURE 6.—Halo length, seed-surface area, seed volume, and boll volume. Halo length represents the average of 10 seeds with 6 measurements each around the median region of the long axis. Seed-surface area is the average of 25 seeds, seed volume of 40 seeds; and boll volume of 10 bolls.

differential growth rate must be the basis for the physiological explanation of the fiber arrays with all their variations. A study of sorter graphs such as that for 50-day-old bolls in figure 8 suggested to the writers that perhaps each of the 24 to 25 groups may have originated from the seed surface at approximately equal intervals of time, i.e., the longest fibers may have arisen during the first few days of relatively small surface area, the bulk of the fibers represented by the larger blocks may have arisen during the period of rapid expansion in surface area to about 21 days (fig. 6), and the short fibers may have arisen during the period of declining expansion in surface area. Such an explanation is based on the assumption that

there is a continual differentiation of epidermal cells for many days after flowering. An article by Turner (*24*), received at the end of the first season's work, contained evidence of such a continuous differentiation. Gulati (*15*), Singh (*22*), and Farr (*14*) have likewise presented evidence which makes it appear that such is the case, though Balls (*6*) was not convinced by the evidence of Turner or Gulati and still holds that his earlier statements are probably correct. Barritt (*7*, *8*) also is not convinced that there is a continuous differentiation of epidermal cells.

In 1930, efforts were directed chiefly to obtaining evidence that might refute or substantiate the assumption that the different length groups arose from the seed surface at different times, and that factors acting at different stages of boll development might thereby produce the greatest effect on those groups of fibers in the more active phase of elongation with the resultant variations to be observed in the sorter arrays.

GROWTH OF THE SEED IN SURFACE AREA AND VOLUME

Fifteen hundred blooms were tagged on 1 day at approximately the same branch and node to give material thereafter for 30 daily measurements of seed-surface area, seed volume, and boll volume. The seeds for study were taken from the middle position in the locules of 10 bolls which were collected daily. The fiber was removed, and the length and breadth of the seed measured. The removal of the fiber was difficult for the first few days, but soon it could be performed so that the volume displacements in paraffin oil were fairly accurate. The cottonseed is practically a prolate spheroid, and the formula $2\Pi b^2 - 2\Pi \frac{ab}{2} \sin - 1_e$ was used for calculations of surface area, where a is one half of long axis, b is one half of short axis, and e is the eccentricity. The data for seed from 4 to 30 days of age are given in the following tabulation:

Age, in days	Surface area, in square millimeters	Age, in days	Surface area, in square millimeters	Age, in days	Surface area, in square millimeters
[a] 4	16.6	14	137.7	23	220.2
6	25.6	15	142.9	24	225.5
7	33.2	16	177.5	25	238.5
8	41.6	17	166.6	27	232.4
9	47.7	18	196.3	28	232.1
10	54.7	20	201.9	29	232.4
11	78.4	21	223.1	30	241.5
13	101.7	22	217.7		

[a] 25 seed measured each day.

The curve for surface area is shown in figure 6. It is seen that the surface area of the seed at 30 days is 14.5 times that at 4 days, which of itself might suggest an increase in the number of fibers with increasing age. Much of the work of Farr (*14*) was done in the same laboratories on material grown from seed obtained from the same source and planted in adjoining plots which received similar treatment. The measurements for transverse axes of epidermal cells given by Farr in table II of her paper can, therefore, be used to

calculate the number of epidermal cells per seed at various ages. If her figures of 67,400 epidermal cells at 1 day of age are used, the number for the writers' material will be 409,236 at 7 days, 526,670 at 13 days, and 676,121 at 20 days. Though the transverse axis of the epidermal cell is increasing with age, it is not increasing so rapidly as the increase in surface area, as is indicated by these figures and shown in the illustrations of dividing epidermal cells presented by Gulati (*15*) and Farr (*14*). Turner (*24*), in his table I, gives the totals of fiber and ordinary cells on the 1-day-old seed for two Punjab-American cottons as approximately 27,000 and 28,000. The number of fibers approximates 6,000 at 1 day and 25,000 at 28 days. The number of fibers per seed shown in table 4, calculated by a method which is believed to be more accurate, is only 11,807. It should be noted that the numbers of epidermal cells according to the various calculations, which are only approximations, are therefore much greater than the usual count of fibers per seed, indicating that the majority of the cells may finally produce only fuzz. Barritt (*8*) contends that his measurements for increase in surface area of the epidermal cells agree remarkably well with Farr's figure for the increase in surface area of the ovule, and that the evidence from cell measurements thus does not support the possibility of continuous cell division in the epidermis.

The seeds used for surface-area measurements were also used for the determinations of volume. Forty of the delinted seeds were dropped into a calibrated pipette containing paraffin oil, and the displacement noted. The curve in figure 6 gives some idea of the changing relations of surface area for the production of fibers and the volume of material stored in the seed. A comparison of the curves for boll volume and seed volume indicates that after 10 days, when the boll cavity becomes filled with developing seed and lint, the relative increase in volume of the two structures is about the same.

HALO LENGTH

Balls (*3*) and Brown (*9*) have measured the halo lengths of Egyptian cottons, while Hawkins and Serviss (*16*) have measured those of Acala, an American upland, and Pima, an Egyptian-American cotton. The halo length of Super-Seven was taken in order to determine more definitely the period when maximum length was attained. The fibers were measured on one seed from the same position in a lock of each of 10 bolls. The seeds were floated in paraffin oil and an attempt was made to allow the fibers to assume their natural position as nearly as this could be determined. The fibers when combed almost completely surrounded the seed. No large angle of the wings with the longitudinal axis was formed, as shown by Bailey (*2*), and the writers' method, therefore, differed somewhat from that described by him. Twelve measurements were made of the fibers on each seed, 3 around the pointed end, 3 around the large end, and 3 on each side. It is evident from figure 6 that the growth in length was practically complete in 25 days, a period in agreement with that found by Balls (*3*) for Egyptian cotton. Apparently, however, full length in Super-Seven had not been reached in 30 days, as compared with 21 and 24 days for practically complete length in Acala (*16*).

FIBER ARRAYS OF IMMATURE BOLLS

Preliminary but unsuccessful attempts were made to count the number of fibers per seed at daily intervals by the method that Gulati employed in Turner's (*24*) laboratory. Gulati's (Turner (*24*)) observation of the increasing number of fiber cells on the seed surface from 1 to 28 days and Farr's (*14*) observation of the early stages of fiber elongation from epidermal cells, which were apparently daughter cells of recent division, would seem to indicate a distinct probability that the shorter lengths represented in the array actually did arise at later stages than the longest fibers measured as the halo. The assumption previously made (p. 466) that the 24 or 25 length groups of the sorter array may have originated from the seed surface at approximately equal intervals and that each group may have reached its length under the same approximate conditions must, of course, be accepted only as an assumption, for a weight distribution may not be a very accurate indication of the number of fibers in a group. Iyengar and Turner (*19*) show that the length distribution by number of fibers is only approximately represented by the sorter distribution by weight, though the divergence is not great for the varieties of *Gossypium hirsutum* which they investigated. The number-frequency distribution of the fibers of a Texas cotton shown by Clegg (*11*) is quite similar, however, to the weight distributions as presented in this paper. Clegg states that the effective length found from the number-frequency curve differs from that found from the weight frequency by not more than about 3 percent with the usual cottons, on the assumption that there is a constant fiber weight per centimeter. This assumption is not correct for many cottons, as shown by Turner (*24*), Iyengar and Turner (*19*), Campbell (*10*), and the present writers (table 4).

In order to compare the weight-frequency distribution with a number-frequency distribution, the number of fibers per seed was determined. Three hundred fibers from each length group of an array, if so many were available, were weighed and the number of fibers was calculated for each group. This was done for 11 bolls on one plant (table 3). The array from this number-frequency distribution is compared with the weight-frequency distribution in figure 7. It is seen that the percentage of fibers in the short lengths, especially two, three, four, and five sixteenths, and even to ten sixteenths, is relatively much greater in the number-frequency distribution than in the weight-frequency distribution. Tips of fibers supposedly break with greater ease than other portions, and broken lengths might account for the greater number of fibers in the short lengths. To test this point, 535 fibers from several lengths were mounted in small groups in paraffin oil and examined microscopically at both ends. One can be fairly certain about broken tips on longer fibers, but there is some difficulty in deciding the condition of fibers two sixteenths of an inch in length by the method employed. Of 257 fibers in the eighteen and twenty-two sixteenths groups, 10.9 percent had broken tips and 14 were unclassified. Of the 165 examined in the two sixteenths group, 39 percent were broken and 24 were unclassified. If the 24 unclassified fibers are placed in the broken group, the percentage is raised from 39 to 53.6. It is entirely possible that portions of the tip less than two sixteenths of an inch are broken and thus lost for an array. The

examination of the long fibers leads to this suspicion, though it is difficult to prove. Not very many of the fibers in the two-sixteenths group appeared to be broken tips, however, for the broad, thicker fibers, characteristic of the shorter lengths were most evident. Whatever may have been the source of the broken fibers, it appears that relatively more of them are in the very short groups, and if these could be distributed into their proper places the weight-frequency and number-frequency histograms would more nearly coincide. Thus it

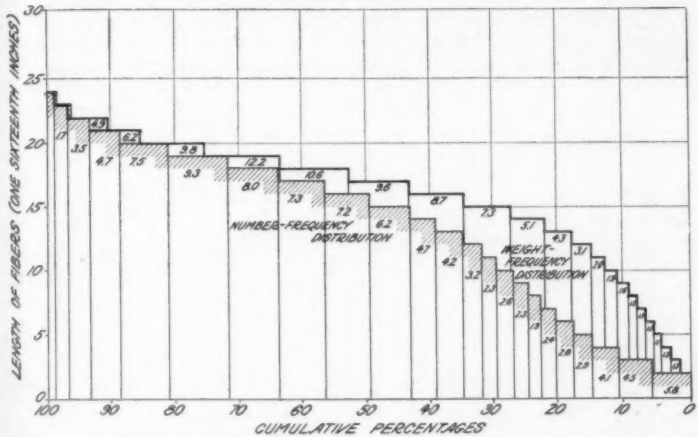

FIGURE 7.—Average weight- and number-frequency distributions of the fibers per seed from 11 bolls of one plant.

appears that the weight-frequency sorter graph can be used as a fair measure of the number of fibers concerned, except perhaps in the very short groups.

TABLE 3.—*Average number of cotton fibers per seed from 11 bolls on 1 plant*

Lengths, in sixteenths of an inch	Average number of fibers per seed	Percentage of total	Lengths, in sixteenths of an inch	Average number of fibers per seed	Percentage of total	Lengths, in sixteenths of an inch	Average number of fibers per seed	Percentage of total
27	0.3	------	17	995.9	7.29	7	331.7	2.43
26	3.9	0.03	16	978.9	7.17	6	378.2	2.77
25	46.4	.34	15	848.8	6.21	5	394.8	2.89
24	113.3	.83	14	638.5	4.67	4	557.2	4.08
23	234.3	1.72	13	572.3	4.19	3	607.7	4.45
22	481.7	3.53	12	437.1	3.20	2	785.7	5.75
21	645.1	4.72	11	312.5	2.29			
20	1,026.5	7.51	10	347.7	2.55	Total	13,661.1	------
19	1,265.1	9.26	9	312.9	2.29			
18	1,089.0	7.97	8	255.6	1.87			

The sorter array of fibers gives an opportunity for counting the number of fibers per seed, but such an array would scarcely be possible at very early stages in boll development. An attempt was made, however, to array fibers at approximately 4-day intervals from bolls 18 to 50 days of age. Ten-boll samples of the different ages were collected at one time. These were air-dried in the laboratory for

2 days and then placed in an oven at very low heat until all that would open had done so. The 50-day-old bolls picked in the morning had cracked by the first afternoon in the laboratory. Fibers from all bolls under 25 days of age were unsatisfactory for an array. Juices flowed from the bolls and the dry mass of fibers crumbled when handled. The fibers from bolls 25 days of age could be prepared for the array with almost the same ease as those from bolls that opened naturally. Fiber arrays for seven samples are represented in figure 8. This figure shows that halo measurements of fibers from bolls 25 days old would be practically the same as those from bolls 50 days old, but that the proportion of fibers 1 inch or longer is only 29.22 percent in the 25-day bolls, whereas in the 50-day bolls it is 80.59 percent.

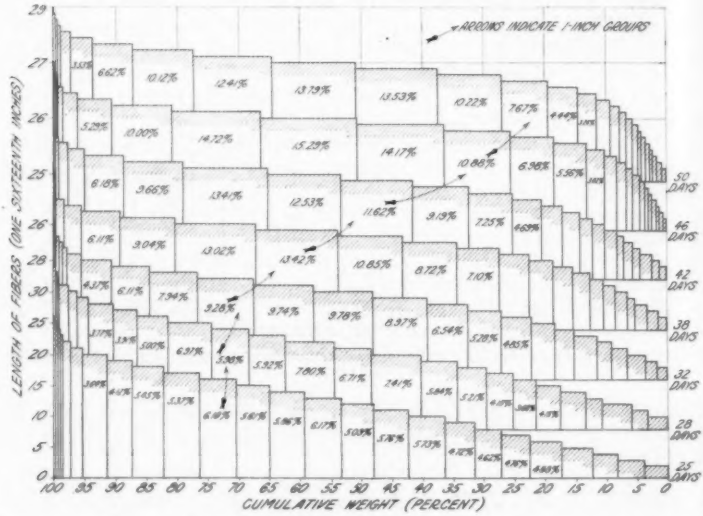

FIGURE 8.—Sorter arrays of the lint from bolls of different ages. Each histogram represents an average of the arrays of 10 bolls. Figures are percentages.

There seemed to be a distinct possibility that more fibers might be broken from a boll 25 days of age than from older ones. Therefore, microscopic observations of 835 fibers from this array, 577 from the array of 38-day-old bolls, and 535 from the array of 46-day-old bolls were made, showing 28, 23, and 22 percent broken fibers, respectively. The relatively small difference in the percentage of broken fibers does not explain the decided differences in the percentages of the total weight in the various length group.

If it is assumed that no further appreciable increase in length of any fibers will occur after 25 days and that the number of fibers per seed is always the same, the change in the distribution of fibers from the 25-day-old boll to that of the 50-day-old boll might conceivably be brought about by a different fiber weight per inch in the process of wall thickening. The curve in figure 6 for fiber growth in length indicates the usual S-shaped growth curve. If it is assumed that some further extension in length of fibers may occur after 25 days, contrary

to the beliefs of Balls (*3*, *4*) and Hawkins and Serviss (*16*), it appears possible that the short fibers which may have arisen at various stages of seed development may continue to grow and reach the final length already attained by others and thereby give the distribution of the 50-day-old boll. If one accepts Balls' (*3*, *4*) statement that the fibers arise on or near the day of blooming, the further extension in length would occur in fibers whose growth had been checked. A changing fiber weight per inch and a further growth in length of some fibers may therefore be important in producing the final result attained. This latter point is the most difficult one on which to secure direct experimental evidence. The unit fiber weight per inch and the number of fibers per seed, however, have been secured for bolls of 25, 32, and 50 days of age. The data for the first and last ages only are shown (table 4).

TABLE 4.—*Number of fibers per seed and the unit fiber weight for cotton bolls 25 and 50 days of age*

Length in sixteenths of an inch	Fibers counted 25 days old	Fibers counted 50 days old	Fibers per seed 25 days old	Fibers per seed 50 days old	Weight per inch of fiber 25 days old	Weight per inch of fiber 50 days old	Ratio of weight, $\frac{50 \text{ days}}{25 \text{ days}}$
	Number	*Number*	*Number*	*Number*	*Milligram*	*Milligram*	
26		19		3.8			
25	48	52	5.3	18.4	0.0013	0.0032	2.46
24	50	130	7.7	83.4	.0013	.0035	2.69
23	50	260	38.8	316.2	.0014	.0040	2.86
22	50	300	77.2	761.8	.0015	.0039	2.60
21	72	300	84.5	1,094.0	.0018	.0038	2.11
20	250	300	163.3	1,282.0	.0024	.0043	1.79
19	286	300	220.5	1,406.4	.0021	.0045	2.14
18	350	300	346.4	1,504.4	.0021	.0049	2.33
17	350	300	426.0	1,109.2	.0023	.0052	2.26
16	350	300	610.6	829.6	.0025	.0054	2.16
15	350	300	661.8	429.2	.0022	.0057	2.59
14	350	300	695.8	400.2	.0023	.0057	2.48
13	350	300	774.3	286.2	.0025	.0059	2.36
12	350	300	663.1	210.6	.0024	.0057	2.38
11	350	300	966.4	187.4	.0025	.0056	2.24
10	350	300	935.2	198.8	.0025	.0058	2.32
9	350	295	830.1	191.4	.0027	.0053	1.96
8	350	300	950.2	111.6	.0028	.0059	2.11
7	350	300	705.5	188.6	.0029	.0063	2.17
6	350	300	694.1	184.2	.0031	.0060	1.94
5	350	300	916.8	204.4	.0031	.0059	1.90
4	350	300	906.6	265.6	.0042	.0076	1.81
3	350	300	1,685.2	231.6	.0043	.0100	2.33
2	350	300	1,449.0	308.0	.0055	.0080	1.45
Total			14,814.4	11,807.0			

The number of fibers per seed for 25- and 50-day-old bolls was obtained from triplicate arrays of the fiber of 9 and 5 seeds, respectively, from 3 different bolls. A total of 350 fibers from each length, if available, was counted for the 25-day-old bolls, and 300 fibers from each length for the 50-day-old bolls. The average number of fibers per seed for the ages 25, 32, and 50 days was 14,814.4, 14,266, and 11,807, respectively, thus showing considerable variation without an indication of an increasing number of fibers per seed after 25 days of age. The curve for growth in surface area of the seed shown in figure 6 would not lead one to expect a large increase in the number of fibers, though the increasing surface area to 30 days might produce other fibers.

An examination of the evidence concerning the possibility that a changing fiber weight per inch during development might cause the difference in the fiber arrays from bolls 25 and 50 days of age, as presented in table 4, shows that the fiber weight per inch at 50 days averages 2.23 times the weight at 25 days. To produce such changes as are shown in the sorter arrays, however, it would be necessary for the medium and longer length groups to increase in weight relatively much more than the shorter ones. While such a tendency is indicated by the figures in the last column of table 4, it is in nowise sufficient to produce the marked change noted from the array. Calculations of the fiber weight per inch from a number-frequency distribution rather than a weight-frequency distribution do not materially change the situation.

Since the changing fiber weight per inch for different lengths does not explain the change in arrays of fibers from bolls 25 to 50 days of age, a growth in length of some fibers after 25 days appears a reasonable explanation. This is supported by the extension of the halo length to 30 days, as shown in figure 6.

The inability to count fibers before the seeds are 25 days old leaves the writers without direct evidence concerning the controversial subject of a continuous differentiation of fibers after the day of blooming, and likewise without direct evidence as to the truth or falsity of the assumption that the different length groups of the sorter array may have originated at different intervals. It seems clear from figure 8, however, that contrary to prevailing ideas, there must have been an increase in the length of many fibers in the boll after 25 days of age.

Because of the evidence for an increasing number of fibers per seed with increasing age to about 28 days (*15*, *24*), the presence of dividing epidermal cells on the outer seed coat (*14*, *15*), fibers of various lengths, side by side at different ages of the seed (*14*, *15*, *22*), increase in surface area of seed greater than increase in length of transverse axes of epidermal cells (*14*), and the finding here reported of an increase in the length of fibers in the boll after 25 days of age, the authors are inclined to believe that this increase in length occurred in fibers which originated from the seed surface at different intervals.

SUMMARY

Cotton fibers from all bolls on individual plants have been arrayed by the use of Baer and Johannsen sorters which allow an accurate separation of the different lengths into groups by weight.

A difference of six sixteenths of an inch in the practical staple length of the cotton classer has been found between the fibers of two bolls located rather close together on a plant. Very appreciable differences in the uniformity of distribution of the fibers of various lengths have also been shown.

In terms of the staple length of the cotton classer, a difference of three sixteenths of an inch in length was found between the lint from seed of one locule and the same difference between the lint from bolls developing from blooms of the same day. Five sixteenths of an inch was the difference in length of lint from blooms of two successive days.

Conditions seemed to be favorable for the production of long lint in bolls from all flowers during certain periods of time. During certain

short intervals, however, conditions seemed very unfavorable, as relatively short lint was produced.

The position of the boll along the vertical axis or the horizontal axis does not seem to be of very great importance in determining the length of lint, though there is a distinct tendency for shorter lint to be produced near the top of the plant when grown in the field.

Studies have been made of halo length, seed-surface area and volume, boll volume, number of fibers per seed after 25 days of seed development, unit-fiber weight, and sorter distribution of fibers in bolls at approximately 4-day intervals from 25 to 50 days of age.

The increase in surface area of the seed was most rapid from about 10 to 18 days of age.

The relative increase in seed volume and boll volume was about the same after 10 days, when the boll cavity becomes filled with developing seed and lint.

The halo had practically reached full length in 25 days, though not absolutely so in 30 days.

There was no evidence of an increase in the number of fibers per seed after 25 days.

The unit-fiber weight was not constant, the shorter fibers showing the greatest weight per inch.

The sorter distributions of fibers from bolls at approximately 4-day intervals from 25 to 50 days of age indicate that many of the fibers continue to elongate after the boll is 25 days old.

LITERATURE CITED

(1) Ayyar, V. Ramanatha, and Rao, C. Jagannatha.
1930. variation in lint length in cotton. Agr. Jour. India 25: 42–52, illus.

(2) Bailey, M. A.
1931. the desirability of interchange of details of methods employed in measuring halo lengths at different experiment stations. Empire Cotton Growing Rev. 8: 215–217, illus.

(3) Balls, W. L.
1912. the cotton plant in egypt, studies in physiology and genetics. 202 p., illus. London.

(4) ———
1915. the development and properties of raw cotton. 221 p., illus. London.

(5) ———
1928. studies of quality in cotton. 376 p., illus. London.

(6) ———
1930. growth fluctuations during the development of seed-cotton. Egypt, Min. Agr. Tech. and Sci. Serv. Bul. 101, 15 p., illus.

(7) Barritt, N. W.
1929. the structure of the seed coat in gossypium and its relation to the growth and nutrition of the lint hairs. Ann. Bot. [London] 43: [483]–489, illus.

(8) ———
1932. the differentiation of the epidermal layer in cotton seed and its relation to ginning out-turn. Empire Cotton Growing Rev. 9: 126–131, illus.

(9) Brown, C. H.
1929. the effect of locality on the halo length of various strains of egyptian cotton. Egypt, Min. Agr. Tech. and Sci. Serv. Bul. 84, 3 p., illus.

(10) Campbell, R. C.
1929. cotton fiber studies. Ga. Agr. Expt. Sta. Bul. 158, 15 p., illus.

(11) Clegg, G. G.
1932. The stapling of cottons. Laboratory methods in use at the Shirley Institute, 1931. Jour. Textile Inst. 23: T35–T54, illus.
(12) Eaton, F. M.
1931. Early defloration as a method of increasing cotton yields, and the relation of fruitfulness to fiber and boll characters. Jour. Agr. Research 42: 447–462, illus.
(13) Ewing, E. C.
1918. A study of certain environmental factors and varietal differences influencing the fruiting of cotton. Miss. Agr. Expt. Sta. Tech. Bul. 8, 93 p., illus.
(14) Farr, W. K.
1931. Cotton fibers. I. Origin and early stages of elongation. Contrib. Boyce Thompson Inst. 3: 441–458, illus.
(15) Gulati, A. N.
1930. A note on the differentiation of hairs from the epidermis of cotton. Agr. Jour. India 25: 313–316, illus.
(16) Hawkins, R. S., and Serviss, G. H.
1930. Development of cotton fibers in the Pima and Acala varieties. Jour. Agr. Research 40: 1017–1029, illus.
(17) Henry, Y.
1901–2. Le coton, sa culture dans les colonies françaises. Agr. Prat. Pays Chauds 1: [219]–249, [347]–378, [479]–492, illus.
(18) Humbert, E. P., and Mogford, J. S.
1927. Variation in certain lint characters in a cotton plant and its progeny. Tex. Agr. Expt. Sta. Bul. 349, 23 p., illus.
(19) Iyengar, R. L. N., and Turner, A. J.
1930. The weight per inch of fibres of different lengths and the number of fibres per seed for each of the standard Indian cottons. Jour. Textile Inst. 21: T417–T440, illus.
(20) Kearney, T. H., and Harrison, G. J.
1924. Length of cotton fibers from bolls at different heights on the plant. Jour. Agr. Research 28: 563–565, illus.
(21) Kelsick, R. E.
1918. Some observations on the relations of lint length to rainfall. West Indian Bul. 17: 79–82, illus.
(22) Singh, T. C. N.
1931. Notes on the early stages in the development of the cotton-fibre and the structure of the boll and seed. Ann. Bot. [London] 45: 378–380, illus.
(23) Sturkie, D. G.
1930. A study of some of the factors affecting lint development in cotton. Ala. Agr. Expt. Sta. Ann. Rpt. 41: 19.
(24) Turner, A.J.
1929. Ginning percentage and lint index of cotton in relation to the number of cotton fibres per seed the effect of environment on ginning percentage and the determination of unit fibre-weight. Indian Cent. Cotton Com. Tech. Lab. Bul. 18, Technol. ser. 13, 41 p., illus.
(25) Venkataramanan, S. N.
1930. The characters of the cotton boll in relation to its flowering period and position on the plant. Agr. Jour. India 25: 189–205, illus.

EFFECT OF SOIL FERTILITY, BOLL-MATURATION PERIOD, AND EARLY OR LATE PRODUCTION OF BOLLS ON THE LENGTH OF COTTON FIBERS[1]

By G. M. ARMSTRONG, *botanist and plant pathologist*, and C. C. BENNETT, *research assistant, South Carolina Agricultural Experiment Station*

INTRODUCTION

In a previous paper[2] the influence of certain factors on the variability in the length of cotton fibers on individual plants has been presented. The plants to be considered in this paper were grown along with those previously described. The details of plot treatment and other experimental methods are found in the first publication, and need not be repeated here.

EFFECT OF SOIL FERTILITY ON LENGTH OF FIBER

One part of the experimental field was given liberal applications of phosphorus, potash, and nitrogen, and the other part was given none. Otherwise cultural conditions were the same on both. The growth of the plants on the well-fertilized or high-fertility plot was apparently normal, and the plants compared quite favorably with those on other high-yield plots at the experiment station. In 1929 the plants on the unfertilized plot began to show signs of a nitrogen shortage relatively early, and finally the symptoms of an unbalanced nutritional condition were strikingly apparent, the deficiency of potash being most clearly evident. On September 14 an appreciable shedding of leaves was noted, which continued until about the middle of October, when frost caused the loss of all leaves. Some shedding of leaves, though of decidedly lesser proportions, also occurred in the well-fertilized plot.

One hundred blooms opening on August 14 and 15 at the first node of branches 1, 2, and 3 were tagged on each plot. On August 21 a larger number of blooms were tagged at the first node on branches 3, 4, and 5 on the unfertilized plot and at branches 4 and 5 on the fertilized plot.

The average arrays of the fibers from 16 bolls and 10 bolls tagged August 14 and 15 from each of the fertilized and unfertilized plots showed a difference in lint length of less than one sixteenth of an inch, except for some of the shorter fibers (fig. 1, *A*). Average arrays of the fibers from 10 and 25 bolls tagged August 21 from each of the fertilized and unfertilized plots were also made. Since the 10-boll and 25-boll arrays checked closely, only the curves for the latter are shown (fig. 1, *B*).

[1] Received for publication Sept. 9, 1932; issued October, 1933. Technical contribution no. 30 (new series) from the South Carolina Agricultural Experiment Station.

[2] ARMSTRONG, G. M., and BENNETT, C. C. SOME FACTORS INFLUENCING THE VARIABILITY IN LENGTH OF COTTON FIBERS ON INDIVIDUAL PLANTS AS SHOWN BY THE SORTER METHOD. Jour. Agr. Research 47: 447-466. 1933.

The same plots were used in 1929 and 1930, and similar comparisons were made for both years. No greater differences were observed, however, than those shown in figure 1, *A* and *B*. It will be seen that

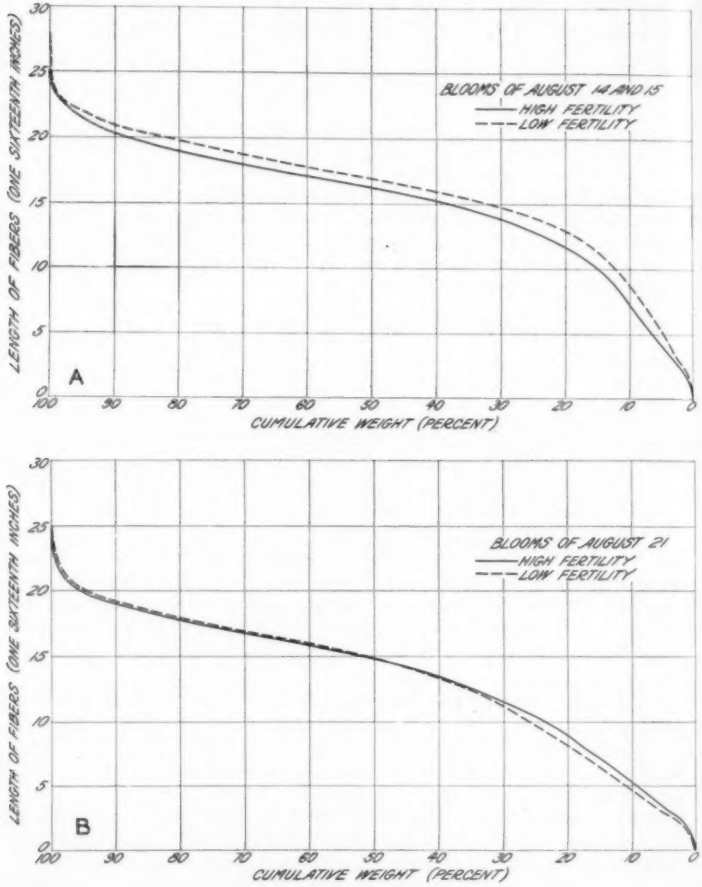

FIGURE 1.—*A*, sorter arrays of the lint from 16 bolls grown on a high-fertility plot and 10 bolls grown on a low-fertility plot; *B*, sorter arrays of the lint from bolls grown on high- and low-fertility plots, each curve representing an average of the arrays of 25 bolls.

the lint length of the cotton grader was practically the same for both samples. The length of the lint was not therefore materially changed by the contrasting treatments, in spite of the very unfavorable conditions for growth in the unfertilized plots, which resulted in stunted and unhealthy plants often bearing only 1 or 2 bolls. Sturkie [3] states

[3] STURKIE, D. G. A STUDY OF SOME OF THE FACTORS AFFECTING LINT DEVELOPMENT IN COTTON. Ala. Agr. Expt. Sta. Ann. Rpt. 41: 19. 1930.

that length of lint and size of boll were the same from plants grown under optimum moisture conditions on a fertile Mississippi Delta soil and a Norfolk sand.

In 1929 all the bolls in this particular phase of the work were marked with India ink and measured twice daily with calipers. The diameter measurements were converted into volume on the assumption that the boll is a sphere, the formula $V=4.189r^3$ being used. Volume-displacement measurements made on 10-boll samples show that the calculated volumes are about 5 and 6 percent less than the actual volumes until the boll is 7 to 9 days old and that the divergence increases thereafter until the calculated volumes are 13 to 16 percent less when the boll reaches full size. A volume measurement based on a 10-boll sample is only approximate, and because of the difficulties of securing and measuring the large number of bolls necessary for accurate determinations of volume, the calculated volumes are used. In contrast with the results of Sturkie,[4] table 1 shows that there were differences in the size of bolls from well-fertilized and unfertilized plots, the bolls from the latter being smaller. The bolls in this experiment were grown under moisture conditions far from the optimum, as compared with those prevailing in Sturkie's experiments, where boll size was unaffected by low soil fertility. There were days when the plants in the field wilted noticeably during the dry weather. Thus boll size was decreased on the low-fertility plot without materially shortening the lint.

TABLE 1.—*Size of bolls from cotton plants grown on fertilized and unfertilized field plots*

Plot	First series, blooms of Aug. 14 and 15				Second series, blooms of Aug. 21			
	Bolls	Age at maximum volume	Maximum volume	Final volume	Bolls	Age at maximum volume	Maximum volume	Final volume
	Number	*Days*	*Ml*	*Ml*	*Number*	*Days*	*Ml*	*Ml*
Fertilized	16	34	31.62±1.14	30.32±1.14	40	36	32.16±0.66	31.04±0.64
Unfertilized	13	33	26.48± .90	23.24± .88	71	37	28.72± .45	27.05± .47

Statistical analyses of boll-size measurements in connection with other phases of the work have shown that results based on 13 or 16 bolls alone should be accepted as doubtful, but the results presented in table 1 for the first series agree with those of the second series with its larger number of bolls and are believed to be indicative of the true situation. A 50-boll sample measured for size gives fairly reliable results, though for a greater degree of accuracy a 100-boll sample should be used.

EFFECT OF AGE OF BOLLS ON LENGTH OF FIBER

It has been noted [5] that bolls on a single plant from blooms of 2 successive days show appreciable variations in length and uniformity of lint, though there is a distinct tendency for lint in all bolls to be

[4] STURKIE, D. G. (See footnote 3.)
[5] ARMSTRONG, G. M., and BENNETT, C. C. (See footnote 2.)

longer or shorter during certain periods. The relation of date of blooming to length of lint was noted in the field in 1929 by tagging 100 blooms at comparable positions on plants in both fertilized and unfertilized plots on August 14 and 15 for the first series and on August 21 for the second series. Fibers from 16 bolls of the first series and 27 bolls of the second series from the fertilized plot were

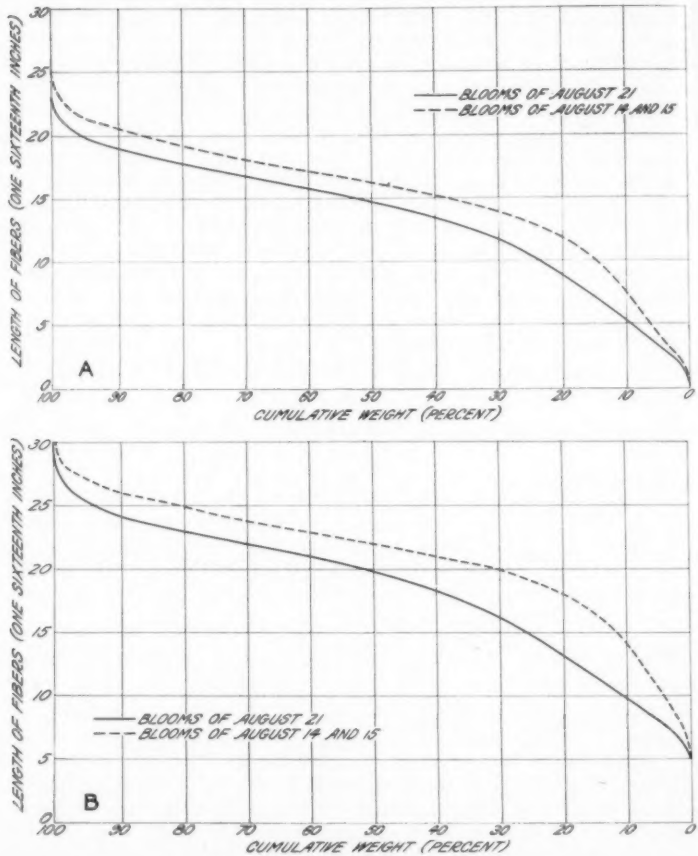
LENGTH OF FIBERS (ONE SIXTEENTH INCHES)
BLOOMS OF AUGUST 21
BLOOMS OF AUGUST 14 AND 15
A
CUMULATIVE WEIGHT (PERCENT)
LENGTH OF FIBERS (ONE SIXTEENTH INCHES)
BLOOMS OF AUGUST 21
BLOOMS OF AUGUST 14 AND 15
B
CUMULATIVE WEIGHT (PERCENT)

FIGURE 2.—*A*, Sorter arrays of the lint from bolls of different ages grown on a high-fertility plot using an average of 16 bolls August 14 and 15, and 27 bolls August 21; *B*, sorter arrays of the lint from bolls of different ages grown on a low-fertility plot using an average of 10 bolls August 14 and 15, and 25 bolls August 21.

arrayed. Curves representing an average of the arrays are shown in figure 2, *A*. It is evident that the percentage distribution of the group lengths has been affected by the age of the bolls and that the later developing fibers are shorter by at least one sixteenth of an inch in terms of the cotton classer. Fiber arrays of 10 bolls of the first series and 25 bolls of the second series from the unfertilized plot are shown

in figure 2, *B*. It is evident that the percentage distribution of the group lengths has been affected by the treatment, for the later developing fibers are almost two sixteenths of an inch shorter in terms of the cotton classer.

That a water shortage may decrease lint length has been shown by Balls [6] and Kelsick [7]. Sturkie [8] states in an annual report, without supporting data, that low soil moisture 1 to 15 days after blooming is associated with short lint. It is also a common belief among cotton growers that the lint length of standard varieties is shorter during dry seasons. An examination of the rainfall and soil-moisture records given in table 2 indicates a probable relation between decreasing soil moisture and the shorter length of lint in the series of August 21. This latter series was subjected to rather low soil moisture for the first 25 days of development, to September 16. At this age bolls have practically reached full size and much of the growth in length of the fibers has been attained.

TABLE 2.—*Rainfall and soil moisture at Clemson College, S.C., for a part of the cotton-growing season of 1929*

Date	Rainfall	Soil depth	Moisture	Date	Rainfall	Soil depth	Moisture
	Inches	*Inches*	*Percent*		*Inches*	*Inches*	*Percent*
Aug. 7	0.02			Sept. 7		6	12.5
9	.05					18	14.5
12	1.00			8	0.19		
17	.10			10	.02		
21		6	2.6	11		6	5.0
		[a] 18	5.1			18	9.0
23	.10			13	.45		
24		6	7.5	14		6	6.9
		18	19.0			18	7.6
27		6	2.9	16	.87		
		18	15.3	17	1.30		
31		6	3.7	22	.34		
		18	12.7	23	Trace		
Sept. 4	.78	6	4.6	25	2.54		
		18	13.0	26	1.30		
6	.02						

[a] 18-inch sample always a heavy clay.

EFFECT OF PERIOD OF MATURATION OF BOLLS ON LENGTH OF FIBER

The date of blooming was used as the basis for the collection of practically all samples because of the fact that some of the most critical stages in boll development occur during the early part of its development and that there are decided differences in the length of the maturation period of different bolls. Burd [9] is of the opinion that bolls which open at about the same time should be used for the comparison of strains of cotton. Balls [10] has shown that the lint lengths are much more irregular if the cotton is gathered by the date of opening of the boll rather than by the date of flowering. Ayyar

[6] BALLS, W. L. THE DEVELOPMENT AND PROPERTIES OF RAW COTTON. 221 p., illus. London. 1915.
[7] KELSICK, R. E. SOME OBSERVATIONS ON THE RELATIONS OF LINT LENGTH TO RAINFALL. West Indian Bul. 17: 79–82, illus. 1920.
[8] STURKIE, D. G. (See footnote 3.)
[9] BURD, L. H. REPORTS ON THE RESEARCH WORK CARRIED OUT AT THE COTTON EXPERIMENT STATION, ST. VINCENT, WEST INDIES. Emp. Cotton Growing Rev. 2: 225–228, illus. 1925.
[10] BALLS, W. L. (See footnote 6.)

and Rao [11] have shown for both *Gossypium hirsutum* and *G. indicum* that definite differences existed in the lint length of seeds produced in different pickings but that there was no definite relation between lint length and the date of picking.

The material from the unfertilized plot offered some distinct contrasts in the maturation period of bolls and two samples have been arrayed. Twenty-four bolls that developed from blooms of the same date and in comparable positions on the plants were selected and divided into two groups of 12 each. The group with the relatively short maturation period contained bolls that opened from 55 to 63 days, the average maturation period being 58 days. The group with the relatively long maturation period contained bolls that opened from 68 to 91 days, the average maturation period being 75.1 days. An inspection of figure 3 shows that the later-opening bolls had more short fibers, and therefore a less uniform staple, but that the practical staple

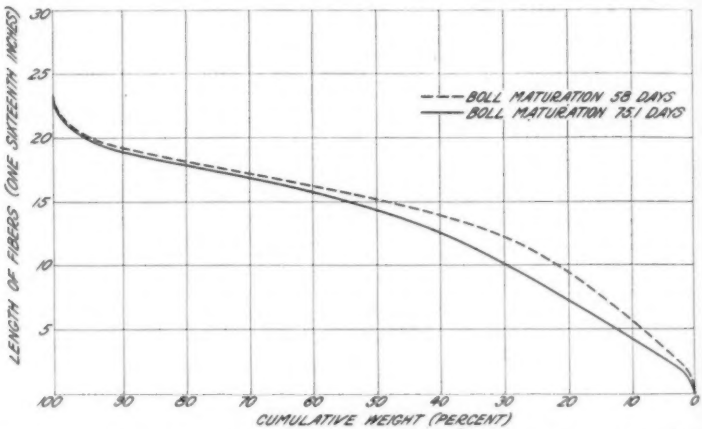

FIGURE 3.—Sorter arrays of the lint from bolls with different maturation periods but arising from flowers of a single day, each curve representing an average of the arrays of 12 bolls.

length was the same. It has been noted previously that the plants on the unfertilized plot were small and that there were very evident signs of unbalanced nutritional conditions, expecially potash hunger, accompanied by some defoliation.

RELATIVE LENGTH OF FIBER IN FIRST AND SECOND CROPS OF BOLLS

The branches and main stems of cotton plants in the greenhouse practically ceased to elongate during the development of the first load of fruit. After most of the first crop of cotton was picked, new growth began at the tips of branches and the main stems, and axillary buds also developed into branches which bore fruit. Since this growth

[11] AYYAR, V. RAMANATHA, and RAO, C. JAGANNATHA. VARIATION IN LINT LENGTH IN COTTON. Agr. Jour. India 25: 42–52, illus. 1930.

in the fall and winter occurred under conditions different from that of the main crop, especially with reference to temperature and length of day, a second crop of bolls was allowed to mature and the cotton was picked chiefly in December, January, and February.

Plant no. 13 produced only 6 bolls as the second crop. The lint from 4 of these would probably be classed as nineteen sixteenths of an inch and that from the other 2 as twenty sixteenths. The lint was neither as long nor as short as that produced in some of the 50 bolls of the first crop. The first crop from plant no. 2 consisted of 20 bolls and the second crop consisted of 11. The average lint length

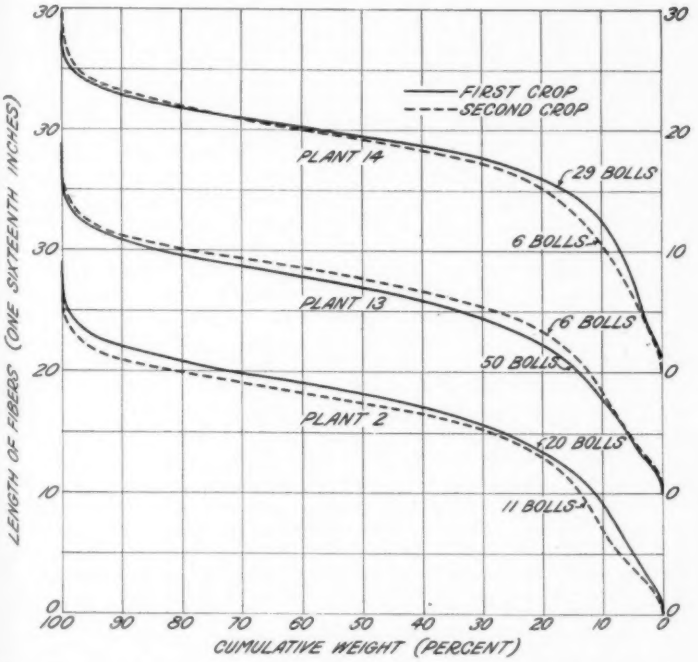

FIGURE 4.—Sorter arrays of the lint from bolls of the first and second crops on plants in the greenhouse.

of the 20 bolls of the first crop was twenty sixteenths of an inch and that of the 11 bolls of the second crop was nineteen sixteenths. The range of variation in lint length from individual bolls of the first crop was seventeen to twenty-one sixteenths of an inch and from bolls of the second crop, seventeen to twenty sixteenths. These illustrations, together with the data presented in figure 4, show that the lint from bolls of the second crop was similar in length to that from bolls of the first crop and that the distributions of the different length groups also maintained about the same relations.

SUMMARY

Small plants, growing on plots of low fertility, and clearly suffering from malnutrition, produced lint of practically the same length as that produced by vigorous plants growing on plots of high fertility, though the uniformity of distribution of the different lengths was less in the poorly nourished plants.

The lint from bolls produced from blooms that were among the last to be retained, namely, those of August 21, was from one sixteenth to two sixteenths of an inch shorter than that from bolls arising a week earlier, on August 14 and 15.

Bolls from blooms opening on the same day but with a maturation period of 75.1 days had a larger percentage of short fibers than those with a maturation period of 58 days, though the practical lint length was the same.

The variability in lint length and the distribution of the different length groups was about the same in bolls of a first crop and in those of a second crop produced on the same plants.

THE NATURE OF THE SHEATH MATERIAL IN THE FEEDING PUNCTURES PRODUCED BY THE POTATO LEAF HOPPER AND THE THREE-CORNERED ALFALFA HOPPER[1]

By FLOYD F. SMITH [2]

Formerly associate entomologist, Division of Cereal and Forage Insects, Bureau of Entomology, United States Department of Agriculture [3]

INTRODUCTION

In a histological study of plant tissue injured by several species of leaf hoppers of the genus *Empoasca*, Smith and Poos (*19*)[4] showed that the feeding of each species was restricted to certain tissues, the mesophyll or the vascular bundle (phloem and xylem), and that the resulting injury to the host was correlated with the parts fed upon. The mesophyll-feeding species of *Empoasca* caused a stippling of the leaves, whereas the species feeding on the vascular tissue, *E. fabae* (Harris),[5] produced severe types of diseaselike injury on its various hosts. In the feeding punctures produced by all species, an amber-colored material that stained bright red with safranine was found either in the form of a cylindrical sheath about the punctures made by the insect's mouth parts or in aggregations at the ends of the punctures. This material was present in both new and old punctures. Smith and Poos suggested that its presence in the vascular cells might interfere with the process of translocation of plant materials and cause the plant to wilt when the xylem vessels were plugged or to become red or yellow when phloem tissue was fed upon.

In order to obtain further knowledge of the injury caused to plants by these insects, particularly by *Empoasca fabae*, it seemed desirable to determine the source of the sheath material and whether it is produced by the plant as a result of the puncture, by the leaf hopper alone, or by both the insect and the plant. The tests were made on sheaths surrounding feeding punctures made by adults of *E. fabae* and also, for comparative purposes, on those made by the adult three-cornered alfalfa hopper, *Stictocephala festina* (Say)[6]. The material was found in greater abundance in its feeding punctures than in those of *E. fabae*. The substances for which tests were made include those that are found in the cell walls and are formed by the plants, as well as additional substances.

REVIEW OF LITERATURE

The literature contains several references to sheaths in the feeding punctures of sucking insects, but investigators have differed in their conclusions as to the nature and source of this material. Prillieux

[1] Received for publication May 31, 1933; issued October, 1933. This paper presents the results of one phase of a cooperative study of leaf-hopper injury to forage crops undertaken by the Division of Cereal and Forage Insects, Bureau of Entomology, and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture.

[2] The writer is indebted to F. W. Poos for making possible the study of this problem; to H. O. Sampson, Mary E. Reid, and H. W. Johnson for advice and suggestions; and to J. W. Schrivener for assistance in preparing the insect-injured material used in the tests.

[3] Now entomologist, Division of Truck Crop and Garden Insects, Bureau of Entomology, U.S. Department of Agriculture.

[4] Reference is made by number (italic) to Literature Cited, p. 484.

[5] Order Homoptera, family Cicadellidae.

[6] Order Homoptera, family Membracidae.

(*17, p. 43*), in his studies of the feeding punctures made by *Puceron* (=*Eriosoma*) *lanigerum* Hausm. in apple in 1878, was apparently the first to describe the sheath in plant tissue around the proboscis of an insect; he believed that the sheath material was analogous to cellulose. Davidson (*4*) stated nearly a half century later that aphid sheaths were probably composed of callose or cellulose and tannin; and more recent tests by Painter (*15, pp. 234–237*) suggested the presence of callose, calcium pectate, and tannins in sheaths caused by punctures of the chinch bug, *Blissus leucopterus* (Say). These results indicate that the sheath was produced by the plant cells. Smith (*20, pp. 118, 120, 131*) believed that the sheath was made up of substances of both plant and animal origin, but he made no tests. King and Cook (*10, p. 8*) came to the same conclusion. Horsfall (*9, p. 9*), working with aphids, thought that the protein material and calcium pectate were laid down by the plant cells in response to the wound stimulus, although he stated (*9, p. 14*) that the protein material might possibly have been ejected by the aphid.

Büsgen (*1, pp. 43–44*), Withycombe (*22, p. 70–81*), Woods (*23, p. 20*), and Zweigelt (*24, p. 274*) concluded that the sheaths were of animal origin. Withycombe and Zweigelt based their conclusions on the fact that they observed the flow of the insect's saliva before or as it made the puncture and this saliva later hardened.

Other observations as to the specific nature of the sheath material are mentioned in the following paragraphs in connection with the substance for which the test was made.

Various workers have employed carbol fuchsin (*23, pp. 20–22*), safranine (*4, pp. 10, 19, 22*), and the haematoxylin stains (*15*) with counter stains for differentiating the sheaths from the plant tissue in histological studies. These stains, although good for permanent mounts, are not satisfactory for quick determinations in fresh material. Several color tests that appear to be desirable differential stains for the sheath material were found during the study reported in this paper.

TECHNIC

Microchemical tests that are described in several available textbooks were used in the present study, and references have been made to these books instead of to the scattered articles originally describing each test. In addition, macrochemical tests were modified for microchemical application. The imperfections of these modifications may account in part for certain inconsistencies in the results, particularly among the tests for the amino acids. Moreover, the negative results obtained with some of the reagents when others used for identification of the same substance were positive may have been due to the insolubility of the sheath in these reagents. Whenever a test was negative, the sheath material was positively identified by testing with Millon's reagent as discussed under the test for tyrosine.

The feeding punctures for study were procured by confining the leaf hoppers in small cages (*18*) to definite areas on petioles, stems, or leaf veins for periods of 1 or 2 days.[7] Alfalfa (*Medicago sativa*), apple (*Malus sylvestris*), cowpea (*Vigna sinensis*), Ladino clover

[7] Fife, in a paper (*5*) that appeared after the completion of the work reported in the present paper, described a feeding cage for use in studies on *Eutettix tenellus* (Baker) by means of which the insect secretion can be obtained apart from the plant tissue and thus interference by the latter in testing for solubility, crystal character, and optical properties can be eliminated.

(*Trifolium repens latum*), red clover (*T. pratense*) var. Tennessee, sweetclover (*Melilotus officinalis*), and potato (*Solanum tuberosum*) were used as host plants.

The tests were made from 1 to 77 days after the insects had been removed from the plants. The tissue was cut with a razor into slices from 1 to 3 cells thick, and these were placed on slides. The sections containing the natural amber or straw-colored sheaths were separated for study, and the tests observed by means of a microscope. Certain solubility tests and others that were prolonged were carried out in small vials, and the observations were made after the material had been transferred to slides.

RESULTS

The results of the tests for each substance, or group of substances, are presented in tables 1 to 3.

PLANT-RESERVE SUBSTANCES

As table 1 shows, the natural color of the sheath material was not changed by either of the dyes Scharlach R or Sudan III, or by osmic acid. The sheath material did not change when subjected to the color tests for tannin or when allowed to oxidize by exposure on a slide for several days. The negative tests in table 1 indicate the absence of plant-reserve substances in sheaths made by the potato leaf hopper and the alfalfa hopper.

So far as the writer knows, tannin is the only material in this group that has been mentioned by previous investigators in connection with sheath material in plant tissue. Petri (*16, p. 28*) stated that tannin was deposited about sheaths in feeding punctures by the grape phylloxera. Davidson (*4, p. 43*) made the same statement with respect to aphids. Painter, working with the chinch bug (*15, p. 234*), and Withycombe (*22, p. 81*) with the sugarcane froghopper (*Tomaspis saccharina* Dist.), said that tannins were always present. None of these investigators, however, mentioned the tests they used.

TABLE 1.—*Tests for plant-reserve substances in the sheath material surrounding punctures in plant tissue made by Empoasca fabae and Stictocephala festina*

Tested for presence of—	Test	Reaction	Reference
Fats and oils	Staining with Sudan III	Negative	Eckerson (*pp. 12–13*).[a]
	Staining with Scharlach R	do	Do.
	Staining with osmic acid	do	Chamberlain (*3, p. 80*).
	Solubility in chloroform	do	Do.
	Solubility in ether	do	Do.
	Solubility in carbon bisulphide	do	Do.
Waxes	Staining with Sudan III	do	Eckerson (*pp. 12–13*).[a]
	Staining with Scharlach R	do	Do.[a]
Gums, mucilage, gelatinized membranes.	Solubility in water	do	Chamberlain (*3, p. 82*).
Tannins	do	do	Gortner (*6, p. 597*).
	Reaction with potassium ferricyanide	do	Do.
	Reaction with 10 percent ferric chloride.	do	Eckerson (*p. 6*).[a]
	Reaction with gold chloride	do	Do.[a]
	Reaction with hydrochloric acid and osmic acid.	do	Do.[a]
	Reaction with ammonium molybdate-ammonium chloride.	do	Withycombe (*22*).
	Oxidation	do	Eckerson (*p. 6*).[a] Gortner (*7, p. 598*).

[a] ECKERSON, S. H. MICROCHEMISTRY. 30 p., n.d. Bot. Dept., Univ. Chicago. [Mimeographed.

Zweigelt (*24, pp. 275–276*), using methylene blue as a test, concluded that tannin was present and that the browning of the sheath on exposure was due to oxidation of the tannic acid present. He also stated that in plants poor in tannin there was no accumulation of this material about the sheaths. This observation is in agreement with the results obtained in the present studies and with those of Wells (*21, p. 280*), who found no tannins in sheaths made by *Pachypsylla mamma* Riley and *P. asteriscus* Riley.

CELL-WALL AND WOUND-RESPONSE SUBSTANCES

In the tests for cell-wall and wound-response substances (table 2) the sheath material did not change color when treated with the reagents giving color reactions for lignin and pentosans or with the solvents copper oxide-ammonia, hydrogen peroxide, or potassium chlorate. Nitric acid colored the sheath material a deep yellow. The chromic acid solution dissolved the heavy lignified walls of the xylem vessels and the sheath material in sections at about the same rate, but much more slowly than it dissolved the remaining plant cells. The similar solubility of lignified cell walls and sheath material in the same sections in chromic acid and their insolubility in copper oxide-ammonia do not necessarily indicate similarity between the two substances, however, for negative results were obtained with the other tests and the chitinous insect exoskeleton shows the same reactions.

Horsfall (*9, p. 9*), using the phloroglucinol test, found no lignin in the sheaths made by aphids.

In the present studies, all tests for callose in the sheath material except the copper oxide-ammonia test were negative. Although the tests employed for its detection are not given, the sheaths made by the grape phylloxera in grape (*16, p. 28*) and of *Macrosiphum rosarum* Walk. in rose (*4, p. 42*) are said to contain this substance, and there are indications that it is also present in the sheaths made by *Blissus leucopterus* in corn and sorghum (*15, p. 234*).

In the tests for cutin and suberin, which were all negative except the test for insolubility in copper oxide-ammonia, the appearance of the sheath material did not change except for the bleaching due to javelle water and Schultze's reagent (ceric acid test). Büsgen (*1, p. 44*) stated that the sheath was converted to a vacuolated mass in Schultze's reagent, but he attached no significance to the results.

All tests for cellulose in the sheath material were negative. In the presence of concentrated sulphuric acid, iodine-zinc chloride solution, hydrocellulose reaction, iodine, or iodine-potassium iodide this material turned brown. Büsgen (*1, p. 44*), using the hydrocellulose reaction, obtained a positive test for cellulose in the outer layer of the sheaths made by *Aphis brassicae* L. and *Coccus cacti* L., but Davidson, although he stated (*4, pp. 42, 52*) that sheaths contained cellulose, obtained negative results with this test and also with copper oxide-ammonia (Schweizer's cupra-ammonia) as a solvent (*4, p. 43*). Büsgen (*1, p. 44*) and Prillieux (*17, p. 43*), using zinc chloriodide, obtained negative tests for cellulose in sheaths. Millardet (*12, p. 8*) mentioned swellings of cellulose following the path of the stylets of the grape phylloxera, but evidently he made no chemical tests.

TABLE 2.—*Tests for plant-cell-wall and wound-response substances in the sheath material surrounding punctures made in plant tissue by Empoasca fabae and Stictocephala festina*

Tested for presence of—	Test	Reaction	Reference
Lignin	Reaction with phloroglucinol-hydrochloric acid.	Negative	Eckerson (*pp. 10, 12*).[a]
	Reaction with orcinol-hydrochloric acid	do	Eckerson (*p. 10*).[a]
	Reaction with Schultze's maceration fluid followed by the hydrocellulose reaction.	do	Chamberlain (*3, pp. 80–81*).
	Insolubility in copper oxide-ammonia	Positive	Eckerson (*p. 12*).[a]
	Solubility in nitric acid	Negative	Do.[a]
	Solubility in hydrogen peroxide	do	Do.[a]
	Solubility in potassium chlorate	do	Do.[a]
	Solubility in 50 percent chromic acid	Positive	Eckerson (*pp. 12–13*).[a]
Pentosans	Reaction with phloroglucinol-hydrochloric acid.	Negative	Eckerson (*p. 10*).[a]
	Reaction with orcinol-hydrochloric acid	do	Do.[a]
	Hydrocellulose reaction	do	Chamberlain (*3, p. 81*).
	Reaction with zinc chloriodide	do	Do.
Callose	Insolubility in copper oxide-ammonia	Positive	Eckerson (*p. 11*).[a]
	Staining with resorcin blue	Negative	Do.[a]
	Staining with aniline blue	do	Do.[a]
	Staining with coralline and sodium bicarbonate.	do	Chamberlain (*3, p. 81*).
Cutin and suberin	Solubility in 1 percent alkali	do	Eckerson (*p. 11*).[a]
	Insolubility in copper oxide-ammonia	Positive	Eckerson (*p. 13*).[a]
	Staining with Sudan III	Negative	Eckerson (*p. 12*).[a]
	Staining with Scharlach R	do	Do.[a]
	Staining with alcannin in 50 percent alcohol.	do	Chamberlain (*3, p. 82*).
	Staining with javelle water and Grübler's cyanin.	do	Do.
	Ceric acid reaction	do	Eckerson (*p. 13*).[a]
	Phellonic acid reaction	do	Do.[a]
	Solubility in 3 percent alcoholic potassium hydroxide.	do	Do.[a]
Cellulose and hemicelluloses.	Hydrocellulose reaction	do	Eckerson (*p. 9*).[a]
	Reaction with zinc chloriodide	do	Hawk (*8, p. 48*).
	Solubility in copper oxide-ammonia	do	Chamberlain (*3, p. 80*).
	Solubility in concentrated sulphuric acid	do	Do.
	Staining with iodine solution	do	Hawk (*8, p. 48*).
	Staining with iodine-potassium iodide solution.	do	Eckerson (*p. 9*).[a]
Pectic compounds	Reaction with phloroglucinol and hydrochloric acid.	do	Gortner (*6, p. 585*).
	Reaction with zinc chloriodide	Positive	Chamberlain (*3, p. 81*).
	Solubility in Schultze's reagent	Negative	Chamberlain (*3, pp. 80, 137*).
	Solubility in ammonium oxalate	do	Gortner (*6, p. 587*).
	Solubility in 2 percent alkalies	do	Eckerson (*p. 12*).[a]
	Staining with ruthenium red	Positive	Eckerson (*pp. 11–12*).[a]
	Staining with methylene blue	do	Do.[a]
Starch, amyloid, saponarin, narceine, dextrin.	Staining with iodine solution	Negative	Eckerson (*p. 9*).[a]
	Staining with iodine-potassium iodide solution.	do	Do.[a]

[a] ECKERSON, S. H. (See footnote to table 1.)

In the tests for pectic compounds the sheath material turned brown, as do pectic compounds, in the presence of zinc chloriodide. Ruthenium red and methylene blue stained the sheath material with their respective colors, a positive test for pectic compounds. Eckerson's procedure was then applied to determine to which class of pectic compounds—pectin, pectose, or pectic acid—the staining was due. An acid-soluble substance behaving like pectose was indicated. Horsfall (*9, p. 9*) and Davidson (*4, p. 43*) obtained similar results by the same procedure in their studies of sheaths made by aphids, but they called the substance calcium pectate. Withycombe (*22, pp. 78–80*) obtained positive staining only occasionally with either ruthenium red or methylene blue after treating with hydrochloric acid. Petri (*16, p. 28*), Wells (*21, p. 280*), and Painter (*15, p. 234*) obtained a positive indication of calcium pectate in sheaths, but they did not give the tests they employed.

14368—33——3

The sheath material did not appear to be reduced in quantity by the solvents for pectic compounds, and it reacted positively to Millon's reagent after such treatment, indicating that the sheath is composed largely of substances other than pectic compounds. If a pectic compound is present, its solubility indicates a pectoselike form. Tests performed on sheaths produced by leaf hoppers feeding from 1 to 22 days previously indicated that the material does not increase in quantity as time passes. If the material is pectose or some other plant substance and is not produced by the insect, it apparently dissolves and intermixes with the insect's secretion, which retains it on hardening. The absence of pectic compounds is evidenced by the staining of the sheath material on the outside of the epidermis at the point of puncture and also in the intercellular and intracellular spaces within the plant tissue.

PROTEINS AND PROTEINÁCEOUS SUBSTANCES

Certain color reactions characteristic of either amino acids or peptide linkages were employed in testing for proteinaceous substances (table 3). Some of these reactions are general protein reactions; others are specific for some particular amino acid.

The sheath material became pink to lilac in the biuret test, indicating the presence of derived proteins, such as proteoses or peptones. Büsgen (*1, p. 44*) obtained a positive biuret reaction in sheaths produced by *Aphis brassicae* and *Coccus cacti*.

A positive xanthoproteic reaction is due to the presence in the protein molecule of benzene nuclei with which the nitric acid forms modifications. The particular complexes of the protein molecule that are of special importance in this connection are those of tyrosine, tryptophane, and phenylalanine. According to Gortner (*6, p. 324*), the benzene nucleus of phenylalanine is not readily nitrated, and the xanthoproteic reaction is specific for aromatic nuclei that are easily nitrated (tryptophane or tyrosine). Sheath material gave positive color reactions for these two materials but did not dissolve. Horsfall (*9, p. 9*) obtained a positive reaction with this test for proteins in sheaths of aphids.

Sheath material, when subjected to the ninhydrin reaction, became a positive greenish-blue color, which indicated the presence of peptones, peptides, or amino acids containing a free carboxyl group and an alpha-amino group.

The negative response to the Molisch reaction indicates that the sheath does not contain a conjugated protein. The negative test with acetic acid-potassium ferrocyanide may have been due to the highly insoluble nature of the sheath material, since the reagent is intended for testing proteins in solution.

Sheath material developed an intense red color [8] when Sakaguchi's test for arginine, as given by Gortner (*6, pp. 325–326*), was made. Only compounds containing the free guanidine group react, but this group is free in most naturally occurring proteins. On sheath material the test is very rapid and it is easy to obtain the color contrast between the sheath and the plant parts. A positive test was obtained at room temperature in from 1 to 2 minutes and then the color faded. Gortner

[8] Since the sheath material was not in solution, 5 cc of water was added to the mixture of sodium hydroxide and alpha-naphthol to make up to the volume of 1 percent protein as recommended.

advises placing the material in the reagent at 2° to 4° C. (35.6° to 39.2° F.) for 40 minutes before examining for color, but slides which the writer prepared and placed on melting ice for 40 minutes showed no color upon examination, the color having faded before the end of this time.

TABLE 3.—*Tests for proteins, amino acids, mucin, and chitin in the sheath material surrounding punctures in plant tissue made by Empoasca fabae and Stictocephala festina*

Tested for presence of—	Test	Reaction	Reference
General proteins	Biuret reaction	Positive	Gortner (*6, p. 323*).
	Ninhydrin reaction	do	Do.
	Xanthoproteic reaction	do	Mathews (*11, pp. 154-155*).
	Molisch reaction	Negative	Gortner (*6, p. 325*).
	Reaction with acetic acid-potassium ferrocyanide.	do	Hawk (*8, p. 103*).
Arginine	Reaction with Sakaguchi's test	Positive	Gortner (*6, pp. 325-326*).
Asparagine and glutamine.	Reaction with quinone solution	do	Eckerson (*p. 19*).[a]
	Reaction with copper acetate and alcohol	Negative	Do.[1]
	Reaction with Ehrlich's reagent	do	Gortner (*6, p. 325*).
	Reaction with Acree-Rosenheim test	do	Do.
	Reaction with Liebermann's test	Positive	Gortner (*6, p. 324*).
Tryptophane	Reaction with Adamkiewicz test	do	Gortner (*6, p. 325*).
	Benedict's modification of Hopkins-Cole reaction.	do	Hawk (*8, p. 98*). Matthews (*11, p. 981*).
	Reaction with Folin and Denis' reagent	Negative	Hawk (*8, p. 86*).
Tyrosine	Reaction with Millon's reagent	Positive	Eckerson (*p. 19*).[a] Mathews (*11, pp. 980-981*).
	Reaction with Folin and Denis' reagent	Negative	Hawk (*8, p. 86*).
	Reaction with Mörner's reagent formaldehyde-sulphuric acid.	do	Morrow (*13, p. 117*).
	Reaction with sodium molybdate-sulphuric acid test.	do	Eckerson (*p. 19*).[a]
Mucin	Staining with thionine blue	do	Mathews (*11, p. 335*).
	Reaction with alkali and Ehrlich's reagent.	do	Mathews (*11, p. 337*).
	Reaction with ferric chloride and hydrochloric acid.	do	Hawk (*8, p. 85*).
	Solubility in concentrated hydrochloric acid.	do	(b).
	Solubility in concentrated potassium hydroxide.	do	(b).
	Solubility in 0.5 percent sodium hydroxide	do	(b).
Chitin	Solubility in 50 percent chromic acid	do	Eckerson (*pp. 12-13*).[a]
	Chitosan reaction	Positive	Eckerson (*p. 13*).[a] Campbell (*2, pp. 404-405*).

[a] ECKERSON, S. H. (See footnote to table 1.)
[b] In tests made by the author, mucin, precipitated from saliva, dissolved in these reagents within 48 hours but sheath material did not.

Sheath material gave a cherry-red color reaction after about 15 minutes in the cold in the presence of quinone solution, indicating the presence of asparagine or glutamine. Chitinous walls of leaf-hopper legs did not give the color reaction, but the muscles and apodemes did. Other tests for differentiation of these amino acids as given by Eckerson [9] were negative. The highly insoluble nature of the sheath material probably prevented any of its components from going into solution and thus accounted for the negative results.

Three of the tests for tryptophane in sheath material were positive, i.e., Liebermann's, Adamkiewicz, and Benedict's modification of the Hopkins-Cole reaction. Benedict's modification of the Hopkins-Cole test produced a reddish-violet coloration of sheath material in plant sections after 10 to 15 minutes in the cold. This test seems to be excellent for color differentiation, since the sheath was the only

[9] ECKERSON, S. H. (See footnote to table 1.)

material in the plant section that took the color and the results were obtained quickly and without distortion of cell structure.

Although the Acree-Rosenheim test for tryptophane gave no color reaction when applied to sheaths or chitinous body walls of leaf hoppers, the vascular tissues in sections from badly injured stems gave positive violet to black colorations, in contrast to uninjured tissue. This indicates that proteins may accumulate, as well as carbohydrates, as previously indicated (*7*), owing to interference in translocation by the feeding of the insects.

In the tests for tyrosine the sheath material became brick red when treated with Millon's reagent. The test is quickly made and the contrast makes the reagent a useful one in detecting feeding punctures.

Büsgen (*1, p. 44*) was the first to report a positive test on sheath material with Millon's reagent and commented on its specific color reaction. Morstatt (*14, pp. 354–355*) was unable to obtain a positive color test with this reagent on sheaths of the scale insect *Diaspis fallax* Horvath. Zweigelt (*24, p. 275*) believed that the positive test obtained with Millon's reagent did not mean that no other substances were included, and Petri (*16, p. 9*, footnote) believed that the test indicated an albumin-tannic acid combination. In some of the sheaths of *Stictocephala festina* the tubular wall seemed to consist of two layers, an inner, dark brick-red, thin-walled layer of rather definite outline and an outer, lighter red accumulation less regular in outline. Painter (*15, p. 234*) observed the two layers in the sheath of the chinch bug. The first evidence of the plugging of xylem vessels by sheath material was obtained by the writer (*19, p. 278*) with this reagent. In tests with Millon's reagent on the chitinous parts of the bodies of *Empoasca*, a similar red color was observed in the veins of the wings and in the spines and walls of the legs. The similarity in the response of the insect exoskeleton and the sheath material secreted by the same insect is extremely interesting. This reaction is given by all organic compounds containing a monohydroxybenzene nucleus, including phenol and salicylic acid (*11, p. 154*). According to Gortner (*6, p. 324*), the red coloration is specific for tyrosine in protein substances.

Sheath material and chitinous insect parts gave negative tests for tyrosine when treated with Folin and Denis' reagent, Mörner's reagent, or sodium molybdate-sulphuric acid. These materials became chestnut brown in the presence of the last two reagents. The vascular tissue in the badly injured sections gave positive blue color reactions when treated with Folin and Denis' reagent or with sodium molybdate-sulphuric acid. The greater brilliance of the blue color observed in treated sections of injured plants as compared with that seen in uninjured tissue of the same age suggests the possibility of local accumulation of proteins in addition to starch (*7*) as a result of insect feeding and the consequent interference with translocation of plant material.

In the tests for mucin in the sheath material, chitinous parts of leaf hoppers and chitosan produced from chitin gave negative reactions when treated with Ehrlich's reagent, ferric chloride and hydrochloric acid, or with thionine blue, as compared with mucin from saliva. These materials did not dissolve in concentrated hydrochloric acid, potassium hydroxide, or in 0.5 percent sodium carbonate, as did mucin.

Horsfall's test with thionine blue for mucin in sheaths of aphids was negative (*9, p. 9*).

Büsgen (*1, p. 44*) and Davidson (*4, p. 43*) stated that javelle water dissolved the sheaths of aphids. In the present study sheath material was dissolved in 1½ hours by several changes of javelle water.

Chitin is relatively insoluble in 50 percent chromic acid. In tests with this reagent on sheaths in plant sections, as indicated under the tests for lignin, all the cells except traces of the xylem tubes dissolved at the end of 22 minutes, but portions of the sheath walls remained. Chitinous walls on legs of leaf hoppers were apparently unchanged by a like treatment, but these parts are much larger than the thin walls of the sheath.

Sheath material, when tested for chitosan, gave a positive delicate violet color. Three percent acetic acid dissolved the sheath material after it had been changed by the chitosan procedure. Upon addition of 75 percent sulphuric acid the violet-colored sheath dissolved as the color faded; then, after a short interval, the cellulose walls of the plant cells exhibited the typical hydrocellulose reaction. With the exception of the test for chitosan sulphate crystals, the chitosan procedure was checked by carrying out the tests upon chitinous body parts of *Empoasca*. A flocculent material in the cells, particularly of the vascular bundle, gave a blue color with iodine-potassium iodide and often interfered with the observations on the sheaths. This was avoided by boiling the sections in 12 percent hydrochloric acid for 10 minutes before heating them in potassium hydroxide. The quantity of material that gave the test for chitosan was much less than that which gave a positive test with Millon's reagent.

These tests indicate that chitin is present in the sheaths of these insects, and, so far as the writer is aware, constitute the first evidence to be reported that this substance is secreted by the salivary glands of an insect.

SUMMARY AND CONCLUSIONS

A study has been made of the chemical nature of the sheaths found in feeding punctures produced by the potato leaf hopper (*Empoasca fabae*) and the three-cornered alfalfa hopper (*Stictocephala festina*). Tests were made for materials that might be derived from the surrounding plant cells and for materials that might be produced by the insects. The results are compared with those of previous workers.

In all tests, the sheaths made by the two species responded similarly.

All tests for plant-reserve, wound-response, and plant-cell-wall substances except the color tests for pectin compounds were negative.

Color tests for mucin were negative, but certain color tests for proteins and amino acids showed that the sheath material is largely proteinaceous.

The sheath material may contain chitin, as it responded similarly to chitinous exoskeleton in a number of tests.

In the course of this study four tests were found which differentiate sheath material from plant tissue by color, namely, Adamkiewicz, Sakaguchi, quinone solution, and Benedict's modification of the Hopkins-Cole reaction.

It appears that the sheath is largely of insect origin and contains no plant substance, with the possible exception of pectose. The highly insoluble sheath, persisting in the vascular tissue, probably interferes

with the normal translocation of plant materials and accounts for the typical plant injuries caused by the feeding of *Empoasca fabae* and *Stictocephala festina*.

LITERATURE CITED

(1) BÜSGEN, M.
1891. DER HONIGTAU. BIOLOGISCHE STUDIEN UND PFLANZEN UND PFLANZENLÄUSEN. 87 p. Jena. (Reprint from Jena Ztschr. Naturw. 25: 1–87.)

(2) CAMPBELL, F. L.
1929. THE DETECTION AND ESTIMATION OF CHITIN; AND THE IRRELATION OF "CHITINIZATION" TO HARDNESS AND PIGMENTATION OF THE CUTICULA OF THE AMERICAN COCKROACH, PERIPLANETA AMERICANA L. Ann. Ent. Soc. Amer. 22: 401–426.

(3) CHAMBERLAIN, C. J.
1924. METHODS IN PLANT HISTOLOGY. Rev. ed. 4, 349 p., illus. Chicago.

(4) DAVIDSON, J.
1923. BIOLOGICAL STUDIES OF APHIS RUMICIS LINN. THE PENETRATION OF PLANT TISSUES AND THE SOURCE OF FOOD SUPPLY OF APHIDS. Ann. Appl. Biol. 10: 35–54, illus.

(5) FIFE, J. M.
1932. A METHOD OF ARTIFICIALLY FEEDING THE SUGAR-BEET LEAFHOPPER. Science (n.s.) 75: 465–466, illus.

(6) GORTNER, R. A.
1929. OUTLINES OF BIOCHEMISTRY, THE ORGANIC CHEMISTRY AND THE PHYSICO-CHEMICAL REACTIONS OF BIOLOGICALLY IMPORTANT COMPOUNDS AND SYSTEMS. 793 p. New York and London.

(7) GRANOVSKY, A. A.
1930. DIFFERENTIATION OF SYMPTOMS AND EFFECT OF LEAF HOPPER FEEDING ON HISTOLOGY OF ALFALFA LEAVES. (Abstract) Phytopathology 20: 121.

(8) HAWK, P. B.
1923. PRACTICAL PHYSIOLOGICAL CHEMISTRY; A BOOK DESIGNED FOR USE IN COURSES IN PRACTICAL PHYSIOLOGICAL CHEMISTRY IN SCHOOLS OF MEDICINE AND OF SCIENCE. Ed. 8, rev., 693 p., illus. Philadelphia.

(9) HORSFALL, J. L.
1923. THE EFFECTS OF FEEDING PUNCTURES OF APHIDS ON CERTAIN PLANT TISSUES. Penn. Agr. Expt. Sta. Bul. 182, 22 p., illus.

(10) KING, W. V., AND COOK, W. S.
1932. FEEDING PUNCTURES OF MIRIDS AND OTHER PLANT-SUCKING INSECTS AND THEIR EFFECT ON COTTON. U.S. Dept. Agr. Tech. Bul. 296, 12 p., illus.

(11) MATHEWS, A. P.
1925. PHYSIOLOGICAL CHEMISTRY, A TEXT-BOOK AND MANUAL FOR STUDENTS. Ed. 4, 1233 p., illus. New York.

(12) MILLARDET, A.
1885. HISTOIRE DES PRINCIPALES VARIÉTÉS ET ESPÈCES DE VIGNES D'ORIGINE AMÉRICAINE QUI RÉSISTENT AU PHYLLOXERA. 240 p., illus. Paris, Bordeaux, [etc.].

(13) MORROW, C. A.
1927. BIOCHEMICAL LABORATORY METHODS FOR STUDENTS OF THE BIOLOGICAL SCIENCES. 350 p., illus. New York.

(14) MORSTATT, H.
1908. UNTERSUCHUNGEN AN DER ROTEN AUSTERNFÖRMIGEN SCHILDLAUS, DIASPIS FALLAX NOV. NOM. HORVATH. Centbl. Bakt. [etc.] (II) 21: 349–365, 408–424, illus.

(15) PAINTER, R. H.
1928. NOTES ON THE INJURY TO PLANT CELLS BY CHINCH BUG FEEDING. Ann. Ent. Soc. Amer. 21: 232–242, illus.

(16) PETRI, L.
1909. ÜBER DIE WURZELFÄULE PHYLLOXIERTER WEINSTÖCKE. Ztschr. Pflanzenkrank. 19: 18–48, illus.

(17) PRILLIEUX, E.
1879. ÉTUDE DES ALTÉRATIONS PRODUITES DANS LE BOIS DU POMMIER PAR LES PIQÛRES DU PUCERON LANIGÈRE. Ann. Inst. Natl. Agron. [Paris] (1877/78) 2: 39–49, illus.

(18) SMITH, F. F.
1931. A NEW TYPE OF INSECT CAGE. Jour. Econ. Ent. 24: 914–916, illus.
(19) ——— and POOS, F. W.
1931. THE FEEDING HABITS OF SOME LEAF HOPPERS OF THE GENUS EMPOASCA. Jour. Agr. Research 43: 267–285, illus.
(20) SMITH, K. M.
1926. A COMPARATIVE STUDY OF THE FEEDING METHODS OF CERTAIN HEMIPTERA AND OF THE RESULTING EFFECTS UPON THE PLANT TISSUE, WITH SPECIAL REFERENCE TO THE POTATO PLANT. Ann. Appl. Biol. 13: 109–139, illus.
(21) WELLS, B. W.
1920. EARLY STAGES IN THE DEVELOPMENT OF CERTAIN PACHYPSYLLA GALLS ON CELTIS. Amer. Jour. Bot. 7: 275–285, illus.
(22) WITHYCOMBE, C. L.
1926. STUDIES ON THE AETIOLOGY OF SUGAR-CANE FROGHOPPER BLIGHT IN TRINIDAD. I. INTRODUCTION AND GENERAL SURVEY. Ann. Appl. Biol. 13: 64–108, illus.
(23) WOODS, A. F.
1900. STIGMONOSE: A DISEASE OF CARNATIONS AND OTHER PINKS. U.S. Dept. Agr., Div. Veg. Physiol. and Path. Bul. 19, 30 p., illus.
(24) ZWEIGELT, F.
1914. BEITRÄGE ZUR KENNTNIS DES SAUGPHÄNOMENS DER BLATTLÄUSE UND REAKTIONEN DER PFLANZENZELLEN. Centbl. Bakt. [etc.] (II) 42: 265–335, illus.

VITAMIN A CONTENT OF BARLEY [1]

By E. H. HUGHES

Associate animal husbandman, California Agricultural Experiment Station

INTRODUCTION

It has been shown by Steenbock, Kent, and Gross [2] that barley, when fed alone, is unable to meet the needs of the young, growing animal. These workers have demonstrated that barley is deficient in inorganic salts and in the fat-soluble vitamins.

Barley, in the great valleys of California, is grown at a lower altitude and under very different climatic conditions than is the case in the Corn Belt. If barley, already deficient in fat-soluble vitamins, particularly vitamin A, be exposed to the extreme temperature of harvest time in California, the content of this vitamin may be still further depressed.

EXPERIMENTAL PROCEDURE AND DATA

To test the vitamin A content of barley and to compare it with that of yellow corn and white corn, three experiments were conducted by the writer. The rat was used as the experimental animal. Results in the first experiment were determined by increased growth as measured by body weight, the development of eye lesions, and other external evidences of vitamin A deficiency. The additional measure of the effects of these diets on the oestrous cycle of the rat was included in the second experiment. Evans and Bishop [3] have shown that the constant appearance of cornified cells in the vaginal smear is a characteristic test for a deficiency of the fat-soluble vitamin A in the diet. A quantitative determination of the vitamin A content of barley compared with that of yellow and white corn, after the method of Smith,[4] was the object of the third experiment.

EXPERIMENT 1

In the first experiment the vitamin A content of barley was compared with that of yellow corn and white corn, because in 1919 Steenbock [5] demonstrated that white corn had very little vitamin A and that yellow corn had sufficient vitamin A for growth and reproduction in the rat.

Stock male rats were used and were fed the diets shown in table 1. The rats were kept in steel cages 15 inches long, 10 inches wide, and 11 inches high, not more than five rats being in each cage. The cages were equipped with a raised wire-mesh bottom. The mesh was of a

[1] Received for publication Apr. 10, 1933; issued October, 1933.

[2] STEENBOCK, H., KENT, H. E., and GROSS, E. G. THE DIETARY QUALITIES OF BARLEY. Jour. Biol. Chem. 35: 61-74, illus. 1918.

[3] EVANS, H. M., and BISHOP, K. S. ON AN INVARIABLE AND CHARACTERISTIC DISTURBANCE OF REPRODUCTIVE FUNCTION IN ANIMALS REARED ON A DIET POOR IN FAT SOLUBLE VITAMIN A. (Abstract) Anat. Rec. 23: 17-18. 1922.

[4] SMITH, M. C. A QUANTITATIVE COMPARISON OF THE VITAMIN-A CONTENT OF YELLOW CORN AND THE GRAIN SORGHUM HEGARI AND YELLOW MILO. Jour. Agr. Research 40: 1147-1153, illus. 1930.

[5] STEENBOCK, H. WHITE CORN VS. YELLOW CORN AND A PROBABLE RELATION BETWEEN THE FAT-SOLUBLE VITAMIN AND YELLOW PLANT PIGMENTS. Science (n.s.) 50: 352-353. 1919.

sufficient size to allow the feces to drop through out of reach of the animals. A supply of distilled water and feed was kept in the cages at all times during the experiment.

TABLE 1.—*Constitution of diets used in experiment 1*

Vitamin-A-free diet		McCollum's diet 1		Diet A		Diet B		Diet C	
Constituent	Percent	Constituent	Percent	Constituent	Percent	Constituent	Percent	Constituent	Percent
Dextrin	70	Whole wheat	67.5	Yellow corn	92	White corn	92	Barley	92
Casein (extracted).	18	Casein (extracted).	15.0	Casein (extracted).	6	Casein (extracted).	6	Casein (extracted).	6
Dried yeast	6	Whole milk powder.	10.0	$CaCO_3$	1	$CaCO_3$	1	$CaCO_3$	1
Agar	2	$CaCO_3$	1.5	NaCl	1	NaCl	1	NaCl	1
Salt mixture (Steenbock 40)	4	NaCl	.8						
		Butter	5.2						

Unextracted casein was used for a few days at the beginning of the experiment before extracted casein was available. The casein was extracted with fast-boiling 95 percent alcohol, air being bubbled through for a considerable part of the time. It was dried in the sun for 15 hours.

The barley, yellow corn, and white corn used were recleaned to insure freedom from weed seeds and other foreign matter and were finely ground in an experimental mill of the hammer type.

The group receiving the vitamin-A-free diet was used as a check to be sure that the extracted casein did not contain vitamin A. The animals on this diet gained in weight for a time, then lost weight rapidly. After losing considerable weight, four were given 2 drops of cod-liver oil daily and made rapid recovery, two were not given cod-liver oil daily and both died. They showed evidence of a vitamin A deficiency.

Growth curves of animals fed the stock diet (McCollum's diet 1) are represented in figure 1, *A*. These experimental animals showed normal growth as compared with other males of the colony.

The yellow-corn diet proved not so efficient for growth (fig. 1, *B*) as did the normal stock diet (fig. 1, *A*). There were no evidence of vitamin A deficiency manifested by any of the animals; while growth was not normal on this diet when compared to those fed McCollum's diet 1, the individuals, as measured by gain in weight, were uniform in their increase.

Rats fed the white-corn diet gained very slowly, and all showed evidences of a deficiency of vitamin A. Animals 563 and 569 were given cod-liver oil, one at the end of 10 weeks, the other at the end of 12 weeks. Both recovered rapidly, as indicated by growth curves (fig. 1, *C*). All other animals fed this diet died except rat 649. This rat never weighed more than 120 g but was still alive at the end of the eighteenth week.

An examination of growth curves (fig. 1, *D*) of animals fed a barley diet indicates a lack of growth as compared to those fed yellow corn (fig. 1, *B*). This was particularly true of animals put on this diet at a weight of 40 g. None of them made as rapid or as consistent growth as did those fed the yellow-corn diet. However, rats 632 and 637

were heavier when they were put on the barley diet, and finally attained a weight of 220 g. An improvement in growth was manifested by rat 568 when from 2 to 3 drops of cod-liver oil was given, after it had shown a gradual decrease in weight for 5 weeks. Some improvement was shown by no. 0 and no. 625. Rat 625 was given yeast, the

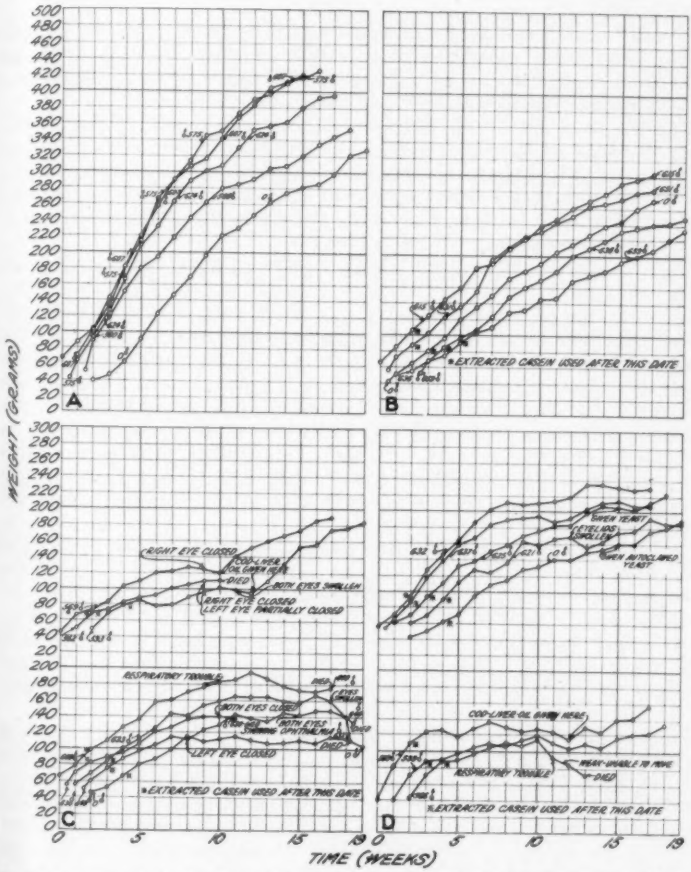

FIGURE 1.—*A*, growth of male rats fed McCollum's diet 1; *B*, growth of male rats fed yellow corn as their sole source of vitamin A; *C*, growth of male rats fed white corn as their sole source of vitamin A, with and without the addition of cod-liver oil; *D*, growth of male rats fed barley as their sole source of vitamin A, with and without the addition of cod-liver oil or yeast.

other autoclaved yeast. No serious ophthalmic disturbances were noticed in those individuals on a barley diet.

When growth is used as a measure of the vitamin A content of yellow corn, white corn, and barley, the indications are that the vitamin A content of barley lies somewhere between that of yellow corn and white corn.

EXPERIMENT 2

In experiment 2, the vitamin A potency of barley as it relates to that of yellow corn and white corn was tested (1) by growth as measured by body weight, and (2) the occurrence of the oestrous cycle as determined by daily vaginal smears. Female rats were used in this experiment.

Evans[6] has shown that a deficiency of vitamin A in the diet results in the constant appearance of cornified cells in the vaginal smear. This is an earlier test of deficiency and probably is more constant in its appearance than are other tests for a lack of this vitamin.

Figures 2, *A* and 2, *B*, represent a complete record of the vaginal smears of rats on the three diets. An additional record of the smears of three individuals from the station normal colony is also shown.

The dashes indicate a period of time in which no smear records were taken. The time in days represents the period in which smears were taken after the opening of the vagina.

Individuals on McCollum's diet 1 showed a regular and normal oestrous cycle.

The vaginae of the animals fed the white-corn diet never opened; therefore, there could be no smear records for them (fig. 2, *A*). The vaginae of rats B1109, W1113, and W1112, fed a barley diet, opened when the rats were about 70 days of age, which was much later than for those fed McCollum's diet 1. Soon after the opening of the vaginae, these rats cycled, W1112 for a period of about 20 days, the other two for a much shorter period. They then showed the cornified cells constantly, until they were fed, for 1 day, a mixture of diets A and B (yellow and white corn); then they began cycling and continued to cycle for a few days. A mixture of equal parts by weight of diets A and B apparently contained a higher potency of vitamin A than did the barley diet. Rat B1109 then showed a constant smear of cornified cells until death. On the one hundred and fifteenth day, 1 percent wheat-germ oil was added to the diet of rat W1113, and in a few days leucocytes and epithelial cells appeared with the cornified. Not until cod-liver oil was added on the one hundred and fifty-second day was there a loss of the cornified cells in the daily smears.

Two drops of cod-liver oil were given to rat W1112 for 14 days, beginning on the seventy-fifth day. This individual began to cycle and continued until the end of the experiment. The addition of 1 percent of wheat-germ oil on the one hundred and twenty-fifth day seemed to depress the cycle. This individual did not cycle so continuously as before, until cod-liver oil was again added on the one hundred and sixtieth day.

Vaginal-smear records of other rats on a barley diet and of three on a yellow-corn diet are presented in figure 2, *B*. Individuals W1103 and W1106 (yellow-corn diet) were very regular and uniform in their cycling. W1110 was not so regular, particularly after the one hundred and eighty-fifth day. The records of the rats on the barley diet are very similar to those shown in figure 2, *A*. Each began cycling after a day's feeding of diets A and B mixed. This was followed by a constant cornified-cell smear until either cod-liver oil or wheat-germ oil was added. Rat G1096 received cod-liver oil

[6] EVANS, H. M. THE EFFECTS OF INADEQUATE VITAMIN A ON THE SEXUAL PHYSIOLOGY OF THE FEMALE. Jour. Biol. Chem. 77:651-654. 1928.

on the forty-fourth day and began cycling on the forty-sixth day and continued to cycle until the end of the experiment. Rats W1108 and B1100 dropped to dioestrus immediately upon the addition of

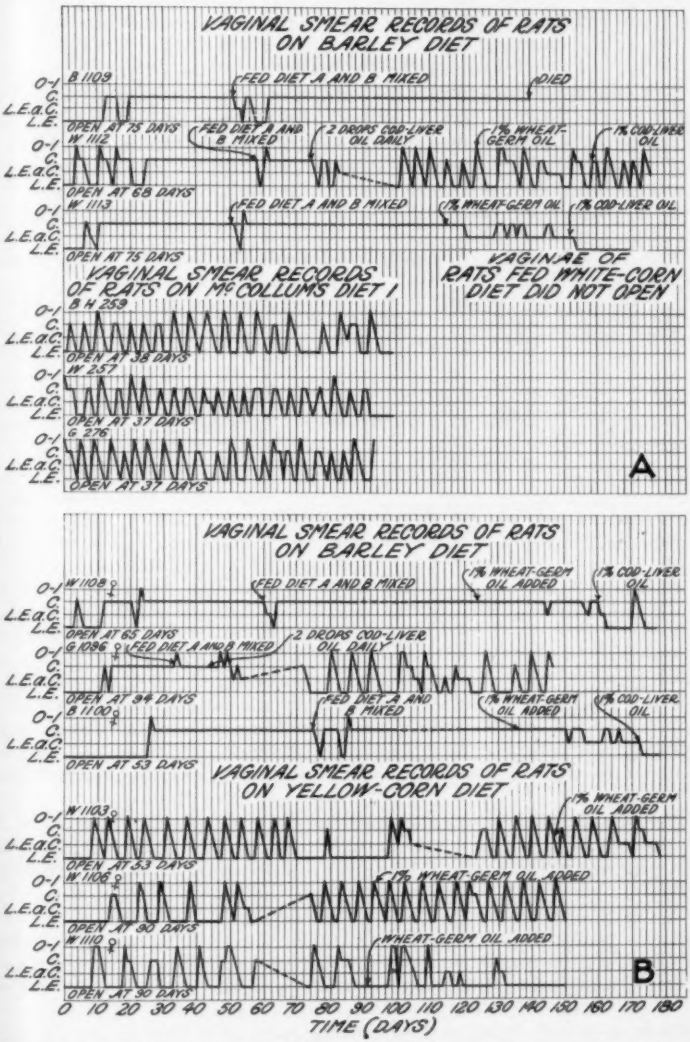
VAGINAL SMEAR RECORDS OF RATS ON BARLEY DIET
B 1109
FED DIET A AND B MIXED
DIED
O-1 C L.E. a C L.E.
OPEN AT 75 DAYS W 1112
FED DIET A AND B MIXED
2 DROPS COD-LIVER OIL DAILY
1% WHEAT-GERM OIL
1% COD-LIVER OIL
OPEN AT 68 DAYS W 1113
FED DIET A AND B MIXED
1% WHEAT-GERM OIL
1% COD-LIVER OIL
OPEN AT 75 DAYS
VAGINAL SMEAR RECORDS OF RATS ON McCOLLUMS DIET 1
VAGINAE OF RATS FED WHITE-CORN DIET DID NOT OPEN
B W 253
OPEN AT 38 DAYS W 257
OPEN AT 37 DAYS G 276
OPEN AT 37 DAYS
A
VAGINAL SMEAR RECORDS OF RATS ON BARLEY DIET
W 1108 ♀
FED DIET A AND B MIXED
1% WHEAT-GERM OIL ADDED
1% COD-LIVER OIL
OPEN AT 65 DAYS G 1096 ♀
FED DIET A AND B MIXED
2 DROPS COD-LIVER OIL DAILY
OPEN AT 94 DAYS B 1100 ♀
FED DIET A AND B MIXED
1% WHEAT-GERM OIL ADDED
1% COD-LIVER OIL
OPEN AT 53 DAYS
VAGINAL SMEAR RECORDS OF RATS ON YELLOW-CORN DIET
W 1103 ♀
1% WHEAT-GERM OIL ADDED
OPEN AT 33 DAYS W 1106 ♀
1% WHEAT-GERM OIL ADDED
OPEN AT 90 DAYS W 1110 ♀
WHEAT-GERM OIL ADDED
OPEN AT 90 DAYS
B
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180
TIME (DAYS)

FIGURE 2.—*A*, vaginal-smear records of female rats fed barley as their sole source of vitamin A, as contrasted with records of female rats fed McCollum's diet 1; *B*, vaginal-smear records of female rats fed barley and yellow corn as their sole source of vitamin A. (O-1 represents epithelial and cornified cells; *C* represents cornified cells; *L. E. a. C.* represents leucocytes, epithelial, and cornified cells; and *L. E.* represents leucocytes and epithelial cells.)

cod-liver oil toward the end of the experiment. Rat W1108 showed one apparently normal cycle just before the conclusion of the experiment. The addition of wheat-germ oil to the extent of 1 percent did

not result in a loss of cornified cells in the smears of rats E1106 and B1100. When growth and vaginal smears are used as a measure of the vitamin A content of barley, it is shown that the quantity in barley is low; in fact, it is apparently much lower than that of yellow corn but is higher than that of white corn.

EXPERIMENT 3

The results of the studies (experiments 1 and 2) indicate that barley is low in its vitamin A content and that its potency lies somewhere between that of yellow and of white corn. Further studies to compare quantitatively the amount of this vitamin in barley and yellow and white corn are reported in experiment 3. The technic and criteria used were those of Smith,[7] being a method developed by Sherman and Munsell.[8]

The yellow corn used was a good grade (at least no. 2) of yellow dent corn purchased locally. White corn of excellent quality (offered for sale as seed corn) was purchased from a seed store. The variety was unknown. Locally grown Yolo County barley of good quality, free from foreign matter and weed seeds, was used.

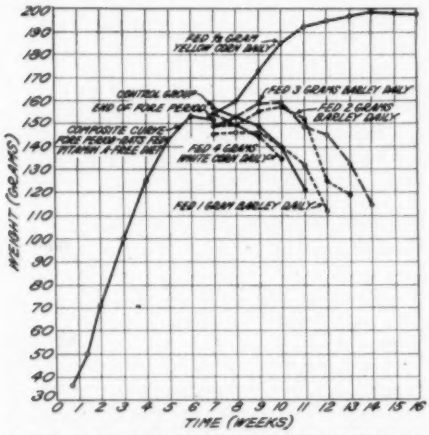
FED 1% GRAM YELLOW CORN DAILY
CONTROL GROUP
END OF FORE PERIOD
FED 3 GRAMS BARLEY DAILY
FED 2 GRAMS BARLEY DAILY
COMPOSITE CURVE FOR FEMALE RATS FED VITAMIN A FREE DIET
FED 4 GRAMS WHITE CORN DAILY
FED 1 GRAM BARLEY DAILY
WEIGHT (GRAMS)
TIME (WEEKS)

FIGURE 3.—Growth records of male and female rats fed a vitamin-A-free diet until the end of the seventh week, designated as the fore period, and thereafter fed the indicated quantities of barley, white corn, and yellow corn daily.

Normal female and male rats from the station experimental colony were used in this series. The females were bred, and during the gestation and lactation periods they were fed Sherman's diet B, except that green alfalfa was given occasionally instead of lettuce. This diet was composed of two thirds ground wheat, one third whole-milk powder, and sodium chloride equal to 2 percent of the wheat.

The young rats from these mothers were weaned at from 20 to 25 days of age and weighed an average of about 37 g each. They were then placed on a vitamin-A-free diet composed of 67 percent of cornstarch, 18 percent of casein (rendered vitamin-A-free by successive extractions with boiling 95 percent alcohol), 10 percent of dried brewer's yeast, 4 percent of salt mixture (Osborne and Mendel's), and 1 percent of sodium chloride. The vitamin D was incorporated by irradiating the dried brewer's yeast, spread out in thin layers, in the direct rays of the sun for 1 hour and 20 minutes.

From weaning time on these young rats were kept in metal cages with screen bottoms. At the end of the fourth week they began showing the effects of the vitamin-A-free diet. A week later some of them were losing weight and showing typical outward symptoms of vitamin A deficiency. Figure 3 shows graphically these weight

[7] SMITH, M. C. Op. cit.
[8] SHERMAN, H. C., and MUNSELL, H. E. THE QUANTITATIVE DETERMINATION OF VITAMIN A. Jour. Amer. Chem. Soc. 47: 1639-1646, illus. 1925.

changes. At the end of the sixth week all had lost weight, or their weight had remained stationary for at least a week.

Each rat was then transferred to a separate cage and fed individually until death, or until the end of the feeding period. Water was provided at all times to each animal. The vitamin-A-free diet was fed in cups and was always available to the individual. The yellow corn, white corn, and barley were weighed and fed daily in small glass dishes. These cereals were very finely ground in an experimental mill of the hammer type.

The 6 groups of 6 rats each were fed the following diets:

Group 1, vitamin-A-free diet (control group)
Group 2, vitamin-A-free diet and ½ g yellow corn daily
Group 3, vitamin-A-free diet and 4 g white corn daily
Group 4, vitamin-A-free diet and 1 g barley daily
Group 5, vitamin-A-free diet and 2 g barley daily
Group 6, vitamin-A-free diet and 3 g barley daily

TABLE 2.—*Average gain or loss in weight and average survival period of animals in experiment 3*

[6 rats in each group]

Group no.[a]	Average initial weight	Average weight, end of fore period	Average first appearance of ophthalmia in rats	Average gain or loss in weight in 56 days	Average survival period
	Grams	*Grams*	*Days*	*Grams*	*Days*
1	37.7	157.0	28.3	−56.3	8.2
2	37.3	153.8	[b] 56.0	+43.3	
3	39.3	153.8	35.5	−29.3	13.2
4	36.3	143.2	37.0	−36.8	17.0
5	38.8	148.3	44.3	−50.0	24.8
6	35.8	148.8	48.2	−39.8	23.0

[a] See fig. 3 and text for diet fed the animals in the various groups.
[b] They were actually carried 68 days before being taken off the diet.

In table 2 and figure 3 are presented the findings in this study. All the animals in group 2 survived and showed no evidence of vitamin A deficiency at any time during the 56 days. Only two other individuals survived. One rat in group 4 died on the fifty-sixth day, and one individual in group 6 lived 67 days after the end of the fore period.

The rats in group 2 were the only ones that showed an average constant gain in weight. An average gain of 43.3 g each for individuals in group 2 indicates that 0.5 g of yellow corn contains more than 1 unit of vitamin A, which is in agreement with the results of Smith.[9] The animals in group 1 (control group), and in group 3, which were fed 4 g of white corn daily, continued to lose weight after the end of the fore period and survived an average of only 8.2 and 13.2 days, respectively. Slight gains were manifested by the experimental animals in groups 4, 5, and 6 for a short period of time after barley was added to the vitamin-A-free diet. Group 4, fed 1 g of barley daily, gained very slightly for 2 weeks, then lost weight rapidly. Groups 5 and 6 gained in weight for 4 weeks, then lost weight rapidly. Group 6, receiving 3 g of barley, gained slightly more than did group 5, fed 2 g of barley daily. None of the rats in groups 4, 5, or 6 survived the 8-week period.

[9] SMITH, M. C. Op. cit.

Three grams of barley fed daily to each individual as the only source of vitamin A was not sufficient to enable the animals to survive the 56-day feeding period. The individuals that received 2 and 3 g of barley each survived somewhat longer than those that received only 1 g of barley daily. All individuals except those fed yellow corn developed ophthalmia at some time before death. Some developed respiratory difficulties. The first appearance of ophthalmia varied somewhat in the various groups; those receiving the 2 and 3 g of barley daily did not show this condition as early as did those fed white corn or 1 g of barley.

All the evidence indicates that barley is low in its vitamin A content when compared with yellow corn. It apparently contains more vitamin A than does white corn. From the facts set forth in figure 3 and table 2 the barley used in this study, which is believed to be an average California barley contained less than one sixth as much vitamin A as did the yellow corn.

SUMMARY

Barley as the only source of vitamin A in the diet did not produce normal growth in rats.

In the sexually immature rat the addition of vitamin A to a barley diet (barley being the only source of vitamin A) resulted in a decided increase in growth and a normal oestrous cycle.

As measured by increase in growth and by daily vaginal-smear record of rats, barley contains more vitamin A than does white corn, but less than yellow corn.

In these studies it has been shown that barley contains less than one sixth as much vitamin A as does yellow corn.

ASSIMILATION OF FIXED NITROGEN BY GRASSES AND CLOVERS [1]

By A. B. BEAUMONT, *head, Department of Agronomy*, W. S. EISENMENGER, *research professor of agronomy*, and W. J. MOORE, JR., *assistant in agronomy, Massachusetts Agricultural Experiment Station* [2]

INTRODUCTION

In the research herein reported certain chemical forms of nitrogen commonly present in commercial fertilizers have been used—nitric, ammoniacal, and amide forms—but no attempt was made to use all the different compounds carrying one or another of these forms of nitrogen. The purpose of these studies was to determine the readiness with which these common forms of nitrogen are absorbed and assimilated by the grasses and clovers usually grown for forage. Methods which involved the growing of plants in nutrient solutions seemed most suitable for the ends sought and were therefore used. Both unsterilized and sterilized culture solutions were used. Each of these methods of growing plants has certain advantages and disadvantages over the other, as will appear later.

METHOD USED WITH UNSTERILIZED CULTURES

Pure seedings of the grasses and clovers used in the experiments were grown in flats. The stands were thick, and on account of the crowded condition of the seedlings and the limited supply of available nutrients (conditions desired in this case), the growth was slow. When about 5 months old, seedlings were transplanted to water cultures, care being exercised to remove as much of the adhering soil as possible. Six seedlings of each grass species, except orchard grass, and four seedlings of orchard grass and each clover species, comprised the individual cultures. There were eight cultures of each treatment. The seedlings were supported by a cork with one hole in it. It was found that plants would remain in place, after some growth, without the aid of cotton which has some disadvantages in such work. Glass bottles of 8-ounce capacity were used. Other details of equipment were similar to those shown in figure 1 of a previous paper (*3*).[3]

A standard nutrient solution containing all nutrients supplied except the nitrogen, and of the following composition was used:

	Mol
$CaHPO_4 \cdot 2H_2O$	0. 00055
$CaSO_4 \cdot 2H_2O$	. 00130
$MgSO_4 \cdot 7H_2O$	. 00285
KCl	. 00188

[1] Received for publication May 22, 1933; issued October, 1933. Published as contribution no. 163 of the Massachusetts Agricultural Experiment Station.

[2] Credit is given G. Rohde and H. Papendieck, formerly assistants in agronomy, for assistance with the solution cultures.

[3] Reference is made by number (italic) to Literature Cited, p. 503.

To this were added 10 parts per million of ferric potassium tartrate, 3 parts per million of boric acid, and the specific nitrogen carrier under consideration. The concentration of sodium nitrate was 0.0139 mol, and ammonium sulphate and urea were used in concentrations to furnish a chemically equivalent amount of nitrogen. The reaction of the fresh nutrient solution was in all cases approximately pH 7.0.

Solutions containing minerals only, sodium nitrate and ammonium sulphate, were changed weekly. This frequency was sufficient to prevent the formation, in detectable quantities, of nitrates in the ammonium sulphate solution. Cultures containing urea (series 1) were changed every 3 days. In the course of the experiment it was found that ammonia was formed from the urea. Therefore, a new urea series (*2*) was set up with a more frequent change of solution. The schedule finally adopted for this series was that of keeping the plants in the solution for 1 day only, changing them to water containing no added salts for 1 day, and then on the third day transferring them to a new nutrient solution. In this way ammonification of urea was either prevented entirely or was reduced to a minimum. Ammonia was occasionally detected in some of the cultures.

There was some variation in the length of the period the plants were grown in the nutrient solutions. The duration of the period was 49 to 50 days for solutions containing minerals only and sodium nitrate; for series 1 urea, the period was 43 days for the grasses, and 49 for clovers; for urea 2, 48 days; for ammonium sulphate, 40 days; and for ammonium sulphate plus calcium carbonate, 37 days. In cases in which the period was less than 48 days the plants were harvested because they had apparently made their maximum growth and were beginning to deteriorate. Dry weights of tops and roots are given in table 1.

TABLE 1.—*Mean dry weight (grams) of various grasses and clovers when grown in different nutrient solutions*

Crop	Mean dry weight of tops						Mean dry weight of roots					
	Check	Sodium nitrate	Ammonium sulphate	Ammonium sulphate plus calcium carbonate	Urea 1	Urea 2	Check	Sodium nitrate	Ammonium sulphate	Ammonium sulphate plus calcium carbonate	Urea 1	Urea 2
Grasses:												
Timothy	0.500	2.51	0.615	0.904	1.97	1.87	0.400	0.364	0.158	0.151	0.210	0.213
Redtop	.323	2.73	.710	1.085	1.31	2.32	.355	.431	.113	.140	.094	.180
Orchard [a]	.462	2.07	.749	.650	1.68	2.03	.351	.240	.096	.095	.278	.246
English ryegrass	.746	2.74	.641	1.046	2.37	2.28	.678	.536	.121	.159	.406	.289
Kentucky bluegrass	.326	1.00	.471	.706	.859	1.41	.253	.270	.053	.108	.118	.339
Mean	.471	2.23	.637	.880	1.64	1.98	.407	.368	.108	.131	.221	.253
Clovers:												
Red	1.92	1.44	.396	.943	2.07	.....	.458	.340	.105	.216	.516	.....
Alsike	1.48	1.55	.241	.694	1.81	3.00	.431	.298	.061	.114	.339	.675
White	.725	1.75	.314	.565	1.85	2.55	.170	.286	.061	.120	.294	.654
Mean	1.38	1.58	.317	.734	1.91	2.78	.353	.308	.076	.150	.383	.665

[a] Only 4 plants grown in each culture. Figures reported are corrected to make results comparable with other grasses, of which 6 plants were grown.

The grass species grown were timothy (*Phleum pratense*), redtop (*Agrostis alba*), Kentucky bluegrass (*Poa pratensis*), orchard grass (*Dactylis glomerata*), English ryegrass (*Lolium perenne*).

The clover species were red (*Trifolium pratense*), Alsike (*T. hybridum*), and white (*T. repens*).

CHEMICAL ANALYSIS OF THE PLANTS

In order to obtain some information regarding changes undergone by the absorbed nitrogen, determinations of total, water-soluble, ammoniacal, and nitric nitrogen were made. It was necessary to combine all the grass species and all the clover species in order to obtain enough material for analysis. This procedure appeared justifiable on account of the marked similarity in the growth behavior of the grasses on the one hand and the clovers on the other. However, it should be noted that some differences in the reaction of the species were observed.

In plant material containing no nitrates, total nitrogen was determined by the official Gunning method (*1*). In determining the water-soluble nitrogen 1.5 g of dry ground material was shaken with 150 cc of water for 1 hour, and 50 cc of the filtrate used for analysis. This was evaporated to dryness on a water bath and the total nitrogen determined by the Gunning method. Ammonia was determined by distillation of 1 g of plant material in 500 cc of water containing 5 g of magnesium oxide, according to the official method (*1*).

In plant material containing nitrates the total nitrogen was determined by the modified method of Ranker (*7*), which involves the reduction of nitrates with sodium thiosulphate. Water-soluble nitrogen was extracted as described above, and then the method described by Pucher and others (*6*) was used. Ammonia was determined by the method described above. Nitrates were determined by the method suggested by Vickery and Pucher (*8*).

Analytical data are given in table 2.

TABLE 2.—*Total and fractional nitrogen content (in percentage of dry weight) of grass and clover cultures grown in different nutrient solutions*

Crop and nitrogen carrier	Tops				Roots			
	Total nitrogen	Water-soluble nitrogen	Ammoniacal nitrogen	Nitric nitrogen	Total nitrogen	Water-soluble nitrogen	Ammoniacal nitrogen	Nitric nitrogen
Grasses:								
Check	0.96	0.28	0.05	0	0.76	0.14	0.13	0
Sodium nitrate	2.97	1.02	.03	.30	2.37	.57	.02	.27
Ammonium sulphate	4.19	2.33	1.33	0	2.63	1.11	.56	0
Ammonium sulphate plus calcium carbonate	4.03	2.16	1.28	0	2.38	.40	.25	0
Urea 1	3.72	1.75	.19	0	2.73	.75	.16	0
Urea 2	2.81	1.19	.13	0	2.46	.67	.16	0
Clovers:								
Check	2.81	.74	.12	0	3.34	.97	.25	0
Sodium nitrate	3.04	1.24	.05	.33	2.89	.94	.07	.41
Ammonium sulphate	3.61	1.74	.75	0	4.25	1.49	.56	0
Ammonium sulphate plus calcium carbonate	4.02	1.99	.55	0	4.06	1.65	.33	0
Urea 1	3.39	1.41	.13	0	4.34	1.76	.21	0
Urea 2	3.53	1.49	.09	0	4.00	1.58	.24	0

REACTION OF THE NUTRIENT SOLUTIONS

The reaction of the nutrient solution before it was used, and without the addition of calcium carbonate, was pH 7.0. The reaction of each treatment was determined colorimetrically at the time of changing solutions; that is, after each growth interval. The data are given in table 3. For solutions carrying ammonium sulphate without calcium carbonate, two pH values are given. The first value represents the end point in the first stages of the experiment when plant growth was vigorous, and the second the end point near the close of the experiment when the plant had practically ceased to function. Grasses which were less susceptible to the conditions present in, or caused by, the solution, both effected a lower pH value and held it lower than did the clovers. The data show interesting relationships for the other solutions. In all the remaining solutions, except ammonium sulphate plus calcium carbonate, the pH values were generally lower for the clovers than for the grasses at the close of each interval. This would indicate the normal physiological effects to be expected. For example, the clover roots were well nodulated and good growth occurred in the check solution without any added nitrogen, and this apparently caused a rapid intake of cations and thus a lowering of the pH values. Likewise, there was less physiological action in the sodium nitrate solution, and consequently less change in the hydrogen-ion concentration of the solution.

TABLE 3.—*Reaction of nutrient solutions at end of changing period, expressed in pH units*

Crop grown	pH of indicated nutrient solution					
	Check	Sodium nitrate	Ammonium sulphate [a]	Ammonium sulphate plus calcium carbonate	Urea 1	Urea 2
Grasses:						
Timothy	6.6	7.8	3.8–6.5	7.1	7.3	7.4
Redtop	6.6	7.7	3.8–6.6	6.9	7.6	7.2
Orchard grass	6.6	7.5	4.0–6.4	7.5	7.3	7.3
English ryegrass	6.6	7.7	5.2–7.0	7.0	7.4	7.3
Kentucky bluegrass	6.5	7.5	4.0–7.0	7.0	7.0	7.0
Clovers:						
Red	5.6	7.0	5.5–7.0	6.9	5.8	6.0
Alsike	5.6	7.0	5.4–7.0	7.0	5.4	6.4
White	5.6	7.1	5.4–7.0	6.9	5.6	6.5

[a] Near beginning and end of the experiment. The first value represents the end point in the first stages of the experiment, when plant growth was vigorous; the second that near the close of the experiment when the plant had practically ceased to function.

DISCUSSION

The data in table 1 clearly show the superiority of sodium nitrate over the other nitrogen carriers used, in the production of dry matter in the grasses. In the case of the clovers, the use of urea increased the dry-matter content most. This holds for both urea 1 and 2, but is especially marked in case of urea 2, in which the solution was changed most frequently and ammonification was at a minimum.

With both grasses and clovers, in all cases ammonium sulphate alone was decidedly the most inferior of all the nitrogen carriers used. The addition of calcium carbonate to the ammonium sulphate culture for the purpose of buffering the reaction, increased the yield over that obtained by the use of ammonium sulphate alone by about 36 percent in the case of the grasses, and about 125 percent in the case of the clovers. Nevertheless, the yield when ammonium sulphate plus calcium carbonate was used was poor in comparison with that obtained when the other carriers were used. Figure 1 also gives a good idea of the growth of the plants in the different media.

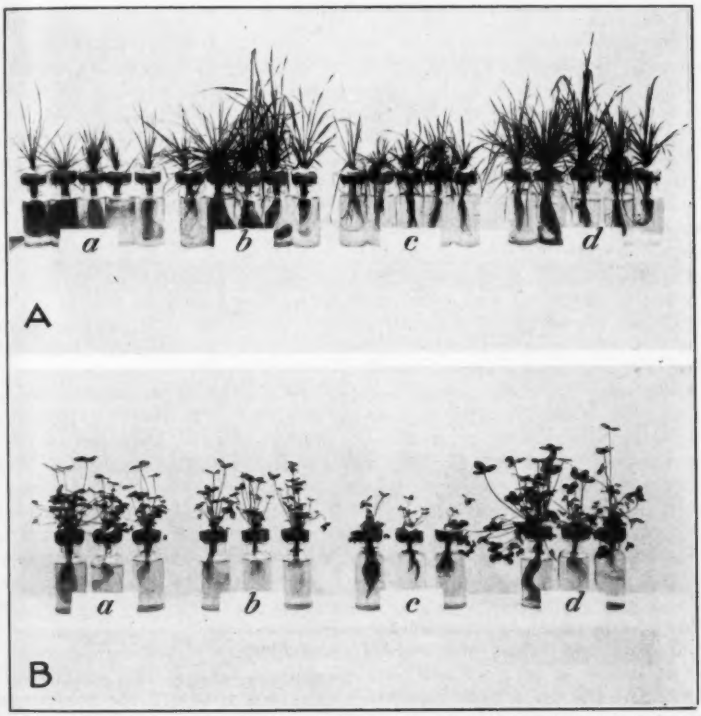

FIGURE 1.—Grasses and clovers grown in unsterilized solutions and photographed when 32 days old. *A*, Left to right in each group: Kentucky bluegrass, English ryegrass, timothy, orchard grass, and redtop; *B*, left to right in each group: Red clover, white clover, and alsike clover. Nitrogen carriers, both series: *a*, Check; *b*, sodium nitrate; *c*, ammonium sulphate (without calcium carbonate); *d*, urea.

The differences recorded in table 1 and figure 1 were those existing at the close of the experiment. In the early stages of the experiment, for about 2 weeks, the growth of the grasses in the solutions containing ammonium sulphate was as good as, if not better than, in other solutions. On the clovers, however, the ammonium salt exerted an inhibiting effect almost from the start. About 2 weeks from the beginning of the experiment it became evident that the roots of both grasses and clovers in ammonium sulphate cultures were not normal in appearance. They became grayish brown and more or less slimy.

Eventually they became dark, and the roots of the clovers, which were more susceptible than the grasses, became almost black. Also, the roots had an odor of decaying organic matter. Practically the same root symptoms developed in the urea culture as in the ammonium sulphate culture, but were more marked in cultures containing urea 1 than in those containing urea 2 because of the greater amount of ammonia in 1. The maintenance of the reaction of the solution containing ammonium sulphate to practically a uniform level by means of calcium carbonate retarded slightly but did not eliminate the toxic action just described.

Table 2 shows that the percentage of total, water-soluble, and ammoniacal nitrogen in the tops and roots of grasses is considerably higher in plants grown in an ammonium sulphate medium than in other media used. The presence of calcium carbonate as a buffer made little difference in this relationship. In the case of the roots, urea caused about as much total and water-soluble nitrogen but not so much ammoniacal nitrogen to enter the plant. This would indicate that some of the urea was present in the roots in an unchanged form. On the grass tops the use of urea 1 produced effects more similar to those produced by the use of ammonium sulphate than did the use of urea 2. This can be explained by the fact that urea 1 solutions were changed less frequently than were those of urea 2, and consequently there was more ammonia present in urea 1; this was shown by chemical test. Nitrates were found only in plants grown in the nitrate solution, and in relatively small amount, which proves that nitrogen supplied in this form was better assimilated than were the other forms of nitrogen used.

According to their assimilability, the forms of nitrogen studied should be placed in the following decreasing order: For grasses: (1) Sodium nitrate (2) urea, and (3) ammonium sulphate; for clovers: (1) urea (2) sodium nitrate, and (3) ammonium sulphate. The order remained the same when the physiological acidity of ammonium sulphate was prevented by the presence of calcium carbonate, although growth was somewhat better under this condition. This leads to the conclusion that under the conditions of the experiment the toxicity of ammonium sulphate is due primarily to the accumulation of excess unassimilated nitrogen in the tissue, particularly the roots of the plant. This conclusion does not preclude the possibility of an injurious effect of the acid produced by physiological action, but it places it in a subordinate position. One of the authors (*2*) arrived at the same conclusion in a previous study of the toxicity of ammonium salts by tobacco.

Other workers in the field of absorption of nitrogen from nutrient solutions have reported decay of roots when grown in media containing ammonium salts. For instance, Loo (*5*) reported this for maize and wheat but attributed it to the physiological acidity of the solutions.

It should be emphasized that the results and conclusions just stated apply only to solution cultures. The writers do not mean to state or imply that the same or closely similar results will be obtained by the use of ammonium sulphate or urea for grasses and clovers grown in soil. Opportunity for rapid nitrification and other salutary actions in the soil appear to make these materials safe and desirable for practical purposes.

POSSIBLE EXPLANATION OF THE DECAY OF THE ROOTS

Series of both grasses and clovers were grown from seeds under sterile conditions according to a method (*4*) previously used for tobacco. The plants were grown 146 days without changing solution, so slow was the growth (fig. 2). Because of certain difficulties encountered it was not possible to obtain quantitative data. The qualitative results obtained checked rather closely those obtained with the unsterilized cultures, with the following exceptions:

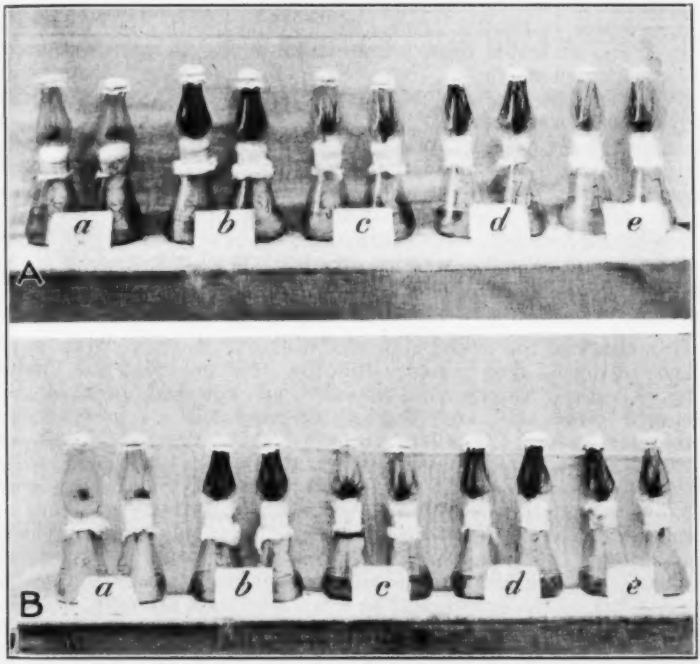

FIGURE 2.—Grasses grown in sterilized solutions and photographed when 146 days old: *A*, Timothy; *B*, redtop. Nitrogen carriers, both series: *a*, Check; *b*, sodium nitrate; *c*, ammonium sulphate; *d*, ammonium sulphate plus calcium carbonate; *e*, urea.

(1) Physiological acidity developed in the unbuffered ammonium sulphate solution and was probably a more important factor than it was in the frequently changed but unsterilized cultures.

(2) Growth was almost, if not quite, as good in the ammonium sulphate solution buffered with calcium carbonate, as it was in the sodium nitrate solution.

(3) Roots of plants grown in cultures containing ammonium sulphate or urea did not turn grayish brown or black and decay, except where it was evident that infection entered from without.

The superior growth in the buffered ammonium sulphate solutions was probably due to the lack of root decay. This is considered a significant point. Under unsterilized conditions common decay

organisms have easy access to the roots, and by their action growth is retarded.

The question then arises as to why the roots decayed in unsterilized solutions containing ammonium salts and urea and did not decay to any serious degree in the check and nitrate solutions. The following hypothesis is advanced:

It has been shown that the nitrogen of ammonium sulphate and urea is not assimilated so completely or so rapidly as that of sodium nitrate. The excess nitrogen accumulates in the plant tissue, especially in the roots. A considerable portion of the excess nitrogen is water-soluble, some of it as ammonia, and probably a fair proportion is in amino or amide form. The carbon-nitrogen ratio is therefore probably much narrowed, especially in the fine roots. It is known from other investigations that organic matter breaks down particularly rapidly when the carbon-nitrogen ratio is narrowed down to about 15:1. It has been proved in the study of methods of making artificial manure from straw that ammonium salts and cyanamide are especially efficacious in promoting the decay of straw. Although these investigations have been with dead plant tissues, it does not seem unreasonable to think that the same method of disintegration of living root cells might occur, especially since some dead root tissue is always present.

SUMMARY

In a study of the assimilability of different chemical forms of fertilizer nitrogen, five grasses—timothy (*Phleum pratense*), redtop (*Agrostis alba*), Kentucky bluegrass (*Poa pratensis*), orchard grass (*Dactylis glomerata*), and English ryegrass (*Lolium perenne*), and three clovers, red (*Trifolium pratense*), alsike (*T. hybridium*), and white (*T. repens*) were grown in nutrient solutions containing sodium nitrate, ammonium sulphate, and urea, respectively. In one group calcium carbonate was added to the solution containing ammonium sulphate for the purpose of buffering it against physiological change in reaction. In the early stages of growth the nitrogen of ammonium sulphate appeared to have been assimilated most rapidly and best by grasses, but in the experiment as a whole nitrate nitrogen was most readily and completely assimilated by the grasses, and urea by the clovers, as determined by the appearance of the plants, weight of dry matter produced, and chemical composition. In the later stages of growth, the plants grown in unsterilized ammonium sulphate solutions and in urea solution developed grayish-brown to black and more or less decayed roots. Plants grown in similar sterilized solutions did not develop this root trouble. It is suggested that the root trouble developed was due to the accumulation in the roots of unassimilated ammonia or other intermediate form of nitrogen, which narrowed the carbon-nitrogen ratio and made the root tissue susceptible to the attack of decay organisms. The order of assimilability of nitrogen is placed as follows: By the grasses: (1) sodium nitrate, (2) urea, and (3) ammonium sulphate; by the clovers: (1) urea, (2) sodium nitrate, and (3) ammonium sulphate. The addition of calcium carbonate to the ammonium sulphate solution did not change the order. The results obtained are not meant to be applied to field conditions under which nitrification and other salutary soil processes may considerably alter the condition of applied nitrogen.

LITERATURE CITED

(1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1925. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. REVISED TO JULY 1, 1924. Ed. 2, 535 p., illus. Washington, D.C.

(2) BEAUMONT, A. B.
1932. THE TOXICITY OF AMMONIUM COMPOUNDS FOR TOBACCO. Second Internatl. Cong. Soil Sci. Proc. (1930) Comn. 4: 65–78, illus.

(3) ——— and LARSINOS, G. J.
1929. A WATER CULTURE TECHNIC FOR STUDIES IN TOBACCO NUTRITION. Jour. Amer. Soc. Agron. 21: 150–155, illus.

(4) ——— LARSINOS, G. J., PIEKENBROCK, P., and NELSON, P. R.
1931. THE ASSIMILATION OF NITROGEN BY TOBACCO. Jour. Agr. Research 43: 559–567, illus.

(5) LOO, T. L.
1931. FURTHER STUDIES ON THE ABSORPTION OF AMMONIA AND NITRATE BY THE ROOT SYSTEM OF THE HIGHER PLANTS. Sun Yatsen Univ., Dept. Biol. Bul. 10, 130 p., illus.

(6) PUCHER, G. W., LEAVENWORTH, C. S., and VICKERY, H. B.
1930. DETERMINATION OF TOTAL NITROGEN OF PLANT EXTRACTS IN THE PRESENCE OF NITRATES. Indus. and Engin. Chem., Analyt. Ed. 2: 191–193.

(7) RANKER, E. R.
1925. DETERMINATION OF TOTAL NITROGEN IN PLANTS AND PLANT SOLUTIONS: A COMPARISON OF METHODS WITH MODIFICATIONS. Ann. Missouri Bot. Gard. 12: 367–380, illus.

(8) VICKERY, H. B., and PUCHER, G. W.
1929. DETERMINATION OF NITRATE NITROGEN IN TOBACCO. Indus. and Engin. Chem., Analyt. Ed. 1: 121–123.

INJURY TO APPLE BY PETROLEUM-OIL SPRAYS[1]

By PAUL A. YOUNG, *assistant botanist and bacteriologist*, and H. E. MORRIS, *botanist and bacteriologist, Montana Agricultural Experiment Station*[2]

INTRODUCTION

Petroleum-oil emulsions were used in attempts to control serious infestations of the fruit-tree leaf roller (*Cacoecia argyrospila* Walker) on apple in Bitterroot Valley of Montana during 1921 to 1925. Some of the emulsions injured the buds and leaves of apple (*Pyrus malus* L.), so it was desirable to secure more information about the effects of petroleum oils on plants, and to learn which oils could be used safely as emulsions on apple. Injury to plants probably is the limiting factor in the use of oil sprays.

This paper reports experiments carried on from 1926 to 1932, at Bozeman, Mont., and records mainly the studies on the character of the injuries to apple from oil sprays.

REVIEW OF LITERATURE

The literature on oil sprays is very extensive. Since Swingle and Snapp (*34*)[3] reviewed many of the articles published before 1929, most attention is given in the present review to more recent papers.

Control of leaf-injuring insects is necessary to prevent decrease in the manufacture of food used by the apple trees. Proebsting (*25*) and Spuler, Overley, and Green (*33*) stated that decreasing the leaf area decreased growth in apple.

Oil emulsions commonly are classified as stable emulsions in contrast to unstable or quick-breaking emulsions in which the oil globules quickly accumulate as cream or free oil at the top of the water phase. In a miscible oil, the emulsifier is dissolved in the oil and makes the oil emulsify when stirred with water. Most of the soap emulsions and the emulsions made from miscible oils for commercial use have been stable emulsions. Casein has been used extensively in making unstable emulsions.

Writers disagree concerning the importance of stability as a quality of spray emulsions. Smith (*30*) favored the use of unstable emulsions. Swingle and Snapp (*34*) reported no practical difference between the covering powers of emulsions made from stock-oil emulsions and from miscible oils, while Anderson et al. (*1*) said that miscible-oil emulsions seemed to be slightly better in covering power.

Newcomer and Yothers (*21*) reported similar scalecidal effects from unstable caseinate-oil emulsions, stable miscible-oil emulsions, and stable boiled-soap-oil emulsions. Spuler, Overley, and Green (*32*)

[1] Received for publication May 8, 1933; issued October. Paper no. 30 of the Journal series of the Montana Agricultural Experiment Station. Contribution from the Botany and Bacteriology Department.

[2] The authors are indebted to the following oil companies for supplying the oils and information about the viscosities and sulphonatable residues of the oils: Standard Oil Co. of California, Standard Oil Co. of Indiana, Shell Oil Co. and L. Sonneborn Sons, Inc., of New York. Green (*14*)[3] determined the sulphonatable residues of oils 5, 13, 15, 16, 21, and 28.

[3] Reference is made by number (italic) to Literature Cited, 520.

observed that, despite the larger run-off, stable emulsions were as effective as unstable emulsions for insecticides in orchards. Robinson, Fisher, and Spuler (*28*) stated that high pressures used in spraying probably reduced the difference in the sizes of oil globules in stable and unstable emulsions. Spuler (*31*) and Spuler, Overley, and Green (*32*) warned against the building of too-thick deposits of oil by quick-breaking emulsions due to repeated sprayings or to the drifting of sprays by the wind. Green (*14*) reported the ability of one oil film to superimpose itself on another. Smith (*29*) illustrated the mechanism of deposition and accumulation of oil when sprays strike solid objects; this gave an explanation for differences in run-off from different emulsions. Bartholomew (*2*) reported the penetration of oil through both sides of citrus leaves.

Fear has been expressed that continual use of oil sprays might be detrimental to orchards, but evidence does not support this claim. Penny (*24*) reported no cumulative-oil injury in an orange orchard sprayed with oil during nine years. Quaintance, Newcomer, and Porter (*27*) also reported that cumulative-oil injury was not evident in thousands of orchards having eight or more annual sprays of oils on dormant trees.

Sulphonatable residues and viscosities of oils have been reported by many workers to be major factors in affecting oil injuries.

Oils for bud sprays should be 65 to 75 percent unsulphonatable, and have viscosities of 85 to 120 seconds. Robinson, Fisher, and Spuler (*28*) specified that oils for dormant use should have viscosities of 100 to 220 seconds, and unsulphonatable residues of 50 to 85 percent. Oils for summer sprays should have viscosities of 55 to 75 seconds and unsulphonatable residues of 85 percent or more. Newcomer and Yothers (*22*) made similar recommendations for summer oil sprays.

Some authors mentioned other factors affecting oil injuries. Ebeling (*5*) concluded that oil injuries to leaves were proportional to the percentage and heaviness of the oil, and inversely proportional to the amount of spreader and the sulphonation of the oil in the spray. Smith (*29, 30*) showed that uniform applications of quick-breaking emulsions required very thorough agitation.

Spuler, Overley, and Green (*33*) stated that the following factors influenced oil injuries to trees: Temperature, number of sprays, amounts of food and water, abundance of fruit, health, and varietal characteristics. Kelley (*18*) reported that the Grimes Golden apple tree transpired more rapidly and was affected more by oils than was the Jonathan apple tree which had a lower rate of transpiration. Felt (*7*) reported seasonal variation in susceptibility of plants to oil injury. Kelley (*17*) found that all of the oils that he used injured young leaves, while only the heavy oils injured old leaves.

Young (*37*) found that drought increased oil injury. Spuler, Overley, and Green (*33*) concluded that trees suffering from drought or malnutrition were especially injured by oils. Woodworth (*36*) stated that a dry condition of leaves facilitated penetration by oils. Browne (*3*) and Woodhams (*35*) advised that trees should have normal amounts of food and water before being sprayed with oils. Hartzell (*15*) warned that weak trees were especially susceptible to oil injury.

Knight, Chamberlin, and Samuels (*19*), and Spuler, Overley, and Green (*33*) reported that plants recovered more quickly from oils with low viscosities than from oils with high viscosities.

Browne (*3*) considered 32° C. to be near the maximum temperature for safe spraying with oils.

Ginsburg (*10*, *11*, *12*) observed that drops of some oils penetrated the lower sides of apple leaves within 1 or 2 hours, the rates of penetration being inversely proportional to the viscosities, and proportional to the amounts of the oils.

Ginsburg (*11*) tested many emulsions for injurious effects on apple, and mentioned the following symptoms of oil injury: Translucent, brown, and chlorotic leaf spots; early dropping of leaves and fruit; early blooming; altering of time of fruit ripening; russeting and decrease in size of fruit; and adhesion of dirt to oily surfaces.

Ginsburg (*8*, *9*, *12*) reported that oil-sprayed leaves were darker green, contained more chlorophyll, and dropped later than unoiled leaves, probably due to insect control. Penny (*24*) observed that heavy oils stimulated growth.

Woodworth (*36*) gave good suggestions in his classification of the effects of oils on plants. Young [4] (*37*, *38*) described symptoms of oil injuries in apple. Swingle and Snapp (*34*), and Farrar (*6*), described some symptoms caused by oils in orchards.

Spuler, Overley, and Green (*32*) illustrated apple flowers without leaves on twigs which had received a delayed dormant spray with a quick-breaking-oil emulsion, indicating that the oil killed only the leaves in the fruit buds on such twigs in this case.

Overholser and Overley (*23*) found that more than three oil sprays sometimes decreased size of fruit and set of buds. Newcomer and Yothers (*22*) illustrated symptoms caused by a heavy oil in three varieties of apple fruits.

Swingle and Snapp (*34*) summarized some reports on the effects of freezing following oil sprays; 5° C. was recommended as a minimum temperature for spraying. Newcomer and Yothers (*21*) concluded that freezing did not increase injury from oils applied in the spring and that delayed dormant oil sprays were injurious.

Spuler, Overley, and Green (*32*) correlated oil-spray injuries with bud development and recommended that oil sprays should not be applied during the critical period of bud development. Kelley (*17*) suggested that oils may partly break the dormancy of apple trees and thus make them more susceptible to injury by cold.

The uses of Oil Red O and Sudan IV to reveal oils in tissues were described by Proescher (*26*) and Haynes (*16*).

MATERIALS AND METHODS

Earlier work on petroleum oils as insecticides showed that the lubricating oils were more satisfactory than the kerosenes or the crude oils.

In the present investigations, 25 kinds of lubricating oils were tested in emulsions. The sulphonatable residues of these oils ranged from 0 to 41.2 percent; their viscosities ranged from 38 to 244 seconds as determined by the Saybolt universal viscosimeter (table 1).

[4] YOUNG, P. A. SPRAY OIL PENETRATION ON APPLE LEAVES, LIMBS, AND FRUIT. 1929. (Abstract of papers, Amer. Soc. Plant Physiol., Des Moines, Iowa.) [Mimeographed.]

Twenty of the oils were tested as 1-percent oil emulsions. These and three other oils were tested in more concentrated emulsions to reveal symptoms of oil injuries and to determine the margin of safety for each oil.

Calcium caseinate, ammonia casein, and cresoap were the emulsifiers used. An electric stirrer was used in emulsifying the oils in tap water.[5] The emulsions were used on the dates when they were prepared. Calcium-caseinate stock emulsions of one third oil were made by mixing 7.2 g of calcium caseinate in 640 cc of water and emulsifying 320 cc of oil in this mixture. Essentially, this method of making calcium-caseinate emulsions was devised by Burroughs (*3a*).

Ammonia-casein stock emulsions were made by mixing 1,500 cc of water and 22.32 g of casein, making this mixture alkaline with about 25 cc of NH_4OH (specific gravity, 0.9), and emulsifying 3,000 cc of oil in it. Spuler, Overley, and Green (*32*) mentioned ammonia-casein-oil emulsions.

Each cresoap-stock miscible oil was made by mixing 90 percent oil, 4.5 percent cresols, and 5.5 percent soap. Potassium oleate was used in preparing cresoap in 1926, and herring oil saponified with KOH was used in 1930 and 1931. For dilution each stock miscible oil was poured slowly on the sides of the vortex made in water by the electric stirrer. The emulsion was agitated for 1 minute after the miscible oil was added. Melander, Spuler, and Green (*20a*) described the method of making cresoap emulsions.

The oil emulsions were sprayed on an orchard of 86 Hibernal apple trees on the farm of the Montana Agricultural Experiment Station at Bozeman. This orchard was planted in 1921 but received little cultural care after 1924 mainly because irrigation water was unavailable. The east row of trees secured some water and food materials from adjacent fallowed ground, and these trees were normal. Many of the trees in the other five rows suffered from drought during each summer and were used in studies on the relation of drought to oil injury.

A bucket pump equipped with a Bordeaux nozzle, and using the maximum pressure, was used to drench apple leaves and twigs with emulsions. Each emulsion was sprayed on all the limbs of sprayed trees in 1926 and 1927; unsprayed trees were left as checks.

Important physiological differences occur in leaves on different sides and levels of apple trees, so, after 1927, single branches 1 to 3 m long were sprayed, and nearby limbs on the same trees were left as checks. Each emulsion was sprayed on 3 to 7 limbs on different trees on each date it was used. Spraying was done on calm days. Summer sprays were applied when temperatures were near 15° to 25° C., maximum temperatures usually near 30° occuring later during the same days. Relative humidities during these days usually ranged between 30 to 70 percent of saturation, with extremes of 14 to 87 percent. Winter sprays were applied when temperatures were about −5° to 10°.

[5] Most of the emulsions used were prepared by J. R. Green, B. L. Herrington, and A. H. Johnson, of the Chemistry Department of the Montana Agricultural Experiment Station.

EXPERIMENTAL RESULTS

MAIN INJURIES CAUSED BY OILS IN APPLE TISSUES

Symptoms appeared in oil-injured leaves within different periods of time. Some symptoms became more prominent, while others remained unchanged or disappeared. Oils with large percentages of sulphonatable residues and oils applied in concentrations of 8, 16, and 100 percent were more injurious than less sulphonatable or concentrated oil emulsions.

Calcium-caseinate-oil emulsions caused angular mottling and translucence in leaves within a few seconds or hours after spraying as the first evidence of injury. These and other emulsions caused chlorotic spots within a few hours or days. In mild injuries translucence and chlorosis did not become more severe and even disappeared.

Severe injuries were characterized by wrinkling, general chlorosis, and hypophyllous [6] browning, silvering, and purpling. Some oils caused epiphyllous purple spots. Cresoap emulsions caused epiphyllous white and tan spots in many leaves.

Translucent and chlorotic spots caused by abundant oils commonly developed into large necrotic brown or dry-green leaf spots, many of which had brown or purple borders. When such spots became very large or numerous, many of the affected leaves fell one to many weeks before the uninjured leaves. Brown spots severely decreased the functions of the leaves. Severe premature defoliation commonly was followed by the dying of many of the buds and some of the bark on attached twigs.

DETAILED DESCRIPTION OF SYMPTOMS CAUSED BY OIL SPRAYS

Oil sprays caused many different symptoms. Brief descriptions and illustrations of some oil injuries are recorded in literature, but as few symptoms have been described in detail, it seems desirable to record the symptoms observed.

The most common symptoms caused by oil emulsions, and the sulphonatable residues and viscosities of the oils that caused them, are given in table 1. It was decided that certain of the oils are too toxic for safe commercial use on apple leaves. The conclusions thus expressed are supported by unpublished data on the effects of undiluted oils on apple.

The comparative degree of injury caused by each oil was determined by the proportion of the injured and the uninjured parts of the sprayed leaves. The degree of oil injury and severity of each symptom is designated by one of the following numbers:

0 means no injury or symptom seen.
1 means traces of injuries and symptoms.
2 means moderate injury and common and prominent symptoms, sometimes economically serious.
3 means severe economic injury and abundant and severe symptoms.
4 means buds killed besides the symptoms included under 3; many of the attached twigs were killed.
– means symptom not considered.

[6] The words "hypophyllous," "epiphyllous," and "amphigenous," have meanings similar to those of the corresponding Latin words as defined by Clements (4). Hypophyllous symptoms occur mainly in spongy parenchyma, and epiphyllous symptoms occur mainly in palisade parenchyma of apple leaves. Most symptoms and many parasites are in tissues instead of on them.

TABLE 1.—*Symptoms of injuries most frequently appearing in apple leaves as a result of spraying with petroleum oils*

Oil no.	Name of oil	Portion sulphonatable	Viscosity	Oil in spray	Duration of test	Sprayed limbs	Usual degree of injury [a]	Premature defoliation [a]	Large, dark brown spots [a]	Epiphyllous purple spots [a]	Epiphyllous white spots [a]	Hypophyllous browning [a]	Chlorosis [a]	Rolling and wrinkling [a]	Oily, angular mottling [a, b]
		Pct.	*Secs.*	*Pct.*	*Yrs.*	*No.*									
3	Standard-Oronite Tech. 13606R	5	100	1	1	17	0	0	1	0	0	0	0	0	0
				1.5	1	340	0	0	0	0	0	2	0	0	0
				2	2	27	0	0	1	0	0	1	1	0	1
				4	3	45	1	1	2	0	2	1	1	0	1
				8	3	33	2	2	3	0	1	2	2	2	2
				16	1	19	4	3	3	0	2	2	2	2	3
4	Standard Mineral Seal 13607R	10	50	1	2	28	0	0	0	0	0	0	0	0	0
				1.5	1	340	0	0	0	0	0	0	0	0	0
				2	2	26	0	0	1	0	1	0	1	0	1
				4	3	44	1	1	1	0	1	1	1	1	1
				8	4	61	2	1	2	0	1	1	2	1	2
5 [c]	Standard 5 no. 14510R	41.6	110	1	1	12	2	2	1	1	1	1	1	2	0
				4	1	13	4	3	2	2	2	1	2	2	1
				8	1	13	4	3	3	1	0	2	2	2	1
6 [c]	Standard Diamond Paraffin	([d])	90	1	1	60	0	0	1	0	0	-	-	-	-
				2	1	60	0	0	1	0	0	-	-	-	-
				3	1	60	2	0	2	0	0	-	-	-	-
7 [c]	Standard Atlantic Red	([d])	244	1	1	60	0	0	0	0	0	0	0	1	0
				2	1	60	1	0	0	0	0	2	1	2	0
				3	1	60	2	1	2	2	0	2	2	2	0
11 [c]	Standard gas oil	40	38	4	1	30	3	2	2	-	-	2	-	-	-
				8	1	30	3	3	2	-	-	3	-	-	-
13 [c]	Shell 70 Pale, RL 99	31.2	75	1	2	35	1	0	1	2	2	2	1	1	0
				2	2	24	2	2	2	2	0	2	1	2	1
				4	3	45	3	3	2	2	2	2	2	2	1
				8	2	40	4	3	2	3	1	2	1	2	-
15 [c]	Shell 2	26.4	58	2	2	24	2	2	2	1	0	2	1	2	1
				4	2	24	3	3	2	2	0	2	1	2	2
16	Shell 3	10	55	1	2	3	0	0	0	0	0	0	0	0	0
				2	2	26	1	1	1	0	0	2	0	0	1
				4	2	27	2	2	2	0	0	2	1	0	1
				8	1	3	2	2	2	0	0	1	0	0	0
18	Shell 5	9.8	108	2	2	26	1	1	2	0	0	1	1	1	1
				4	2	27	2	2	2	0	0	2	1	0	2
				1	1	13	0	0	1	0	0	1	0	0	0
20	Shell 105, H_2SO_4 treated	2	90	2	2	28	0	0	1	0	0	1	1	1	1
				4	3	40	2	2	2	0	0	0	1	2	2
				8	1	12	4	3	3	0	2	2	2	0	2
				1	1	27	0	0	1	0	0	1	0	1	0
21 [c]	Shell 106, SO_2 treated	15.6	53	2	2	27	0	0	0	0	0	2	0	1	1
				4	3	47	3	2	3	1	1	2	2	1	1
				8	2	38	4	3	3	2	1	2	2	2	2
22	Shell 107, H_2SO_4 treated	2	51	1	2	20	0	1	1	0	0	0	0	0	0
				2	2	25	0	0	0	0	0	1	0	0	1
				4	3	41	2	2	2	0	0	1	1	1	2
				8	2	24	3	2	3	0	0	2	1	2	1
23	San Jose emulsion	10	50	1	2	14	0	0	0	0	0	0	0	0	0
				2	2	17	0	0	0	2	0	0	0	0	0
				4	3	27	1	1	2	0	0	0	1	1	1
				8	2	13	2	1	2	0	0	0	1	2	2
24	Sonneborn Amalie 10680P	0	67	1	2	36	0	0	1	0	0	0	0	0	0
				2	2	23	1	1	2	0	0	0	1	0	2
				4	3	44	2	2	2	0	0	0	1	1	2
				8	2	53	3	2	3	0	0	2	0	2	2
				16	2	27	4	3	3	0	0	1	2	1	3
25	Standard 6 no. 14819R	10	73	1	1	20	0	0	0	0	0	0	0	0	0
				2	1	15	1	0	2	0	0	0	0	0	2
				4	2	33	2	2	2	0	0	0	2	0	2
				8	2	36	3	3	3	0	2	2	2	2	2
28 [c]	Standard 7, no. 14776R	35.2	100	1	1	7	1	0	1	0	0	2	0	0	0
				8	1	18	3	3	2	2	1	2	0	0	-
30	S. Ind. Verdol, 67 percent oil	4	83	1	2	13	0	0	0	0	0	0	0	0	0
				4	1	9	2	2	2	0	0	0	1	1	1
				8	2	13	3	3	3	0	0	1	2	0	2
33	Shell E512, SO_2 treated	12.8	52	1	1	5	0	0	0	0	0	0	0	0	0
				8	1	11	3	2	3	0	2	1	0	0	0
34	Shell E513, H_2SO_4 treated	13.3	51	1	1	4	0	0	0	0	0	0	0	0	0
				8	1	10	2	1	2	0	2	1	0	0	0
35 [c]	Shell E514, SO_2 treated	13.7	74	1	1	5	0	0	0	0	0	0	0	0	0
				8	1	12	3	2	2	0	2	1	0	0	0
36	Shell E515, H_2SO_4 treated	14	75	1	1	5	0	0	0	0	0	0	0	0	0
37	Shell E516, SO_2+H_2SO_4 treated	2.3	55	8	1	11	2	1	2	0	2	0	0	0	0
				1	1	5	0	0	0	0	0	0	0	0	0
				8	1	12	2	1	2	0	2	0	0	0	0

[a] See text for explanation of numerical ratings.
[b] Pertains only to calcium-caseinate-oil emulsions.
[c] These oils are not recommended for leaf sprays.
[d] High.

Under the name of oil, "Standard" refers to the Standard Oil Co. of California, except for oil 30, for which it refers to the Standard Oil Co. of Indiana.

Symptoms and Signs of Oil Injuries in Apple Leaves

Premature defoliation means leaf dropping during one to many weeks before normal defoliation (table 1). Very toxic oil emulsions commonly caused serious premature defoliation, usually accompanying and following the development of dark brown spots. Brown leaf spots and premature defoliation were the most common symptoms of severe injuries by oils.

The outer one or two leaves developed by Hibernal apple buds normally were wrinkled and were only half as long as the normal, inner leaves. These dwarfed leaves were especially susceptible to oil injury in 1931, when they turned yellow and dropped from oil-sprayed limbs, while comparable dwarfed leaves on unsprayed limbs remained green and functional during subsequent weeks.

Two-percent oil emulsions of the most toxic oils and more concentrated emulsions of the other oils caused premature defoliation. New leaves developed within 71 days on 19 limbs after the old leaves had been killed by emulsions of 14 very toxic or concentrated oils. Oils did not kill the latent buds on such limbs.

Emulsions of 15 very toxic or concentrated oils killed apple leaves quickly. Abscission layers probably were incomplete in many of such leaves because they became brown and hung on the trees during 1 to 6 months. Many of the twigs died within a year.

Dark brown spots were a common symptom of severe injury and appeared within 5 to 60 days after leaves were sprayed with oils (table 1, and figure 1, *D*). The spots were amphigenous, oval to irregular, and 1 to 30 mm long. Emulsions of the most sulphonatable oils usually caused many brown spots within 5 to 10 days, while emulsions of the least sulphonatable oils more slowly caused few brown spots, and some of them caused no symptoms. Thus, an emulsion of 16-percent oil 24 (unsulphonatable) required 45 days to cause brown leaf spots. These spots became black 36 days later. In contrast, emulsions of 8-percent oil 13 (31.2 percent sulphonatable) caused brown and purple spots within 5 to 19 days.

Brown spots occupied 50 to 90 percent of the blades of badly injured leaves. Some spots were only hypophyllous when they first appeared, but usually later they became epiphyllous too. Oils enlarged many injured spots in leaves.

Dry green spots appeared instead of brown spots in the leaves of many limbs within 11 to 57 days after they were sprayed with 8-percent oil emulsions of nine kinds of oils. Oils in the affected spots probably delayed browning.

The dry green spots commonly had dark-brown or purple borders, irregular lines, or concentric-arc lines 0.5 to 2 mm wide (fig. 1, *D*). Similar borders and lines occurred on many dark brown leaf spots within 12 to 33 days after spraying with 4- and 8-percent oil emulsions in 1930. In some cases the distinctive brown or purple colors of the borders disappeared within 27 days, leaving only the plain brown spots.

14368—33——5

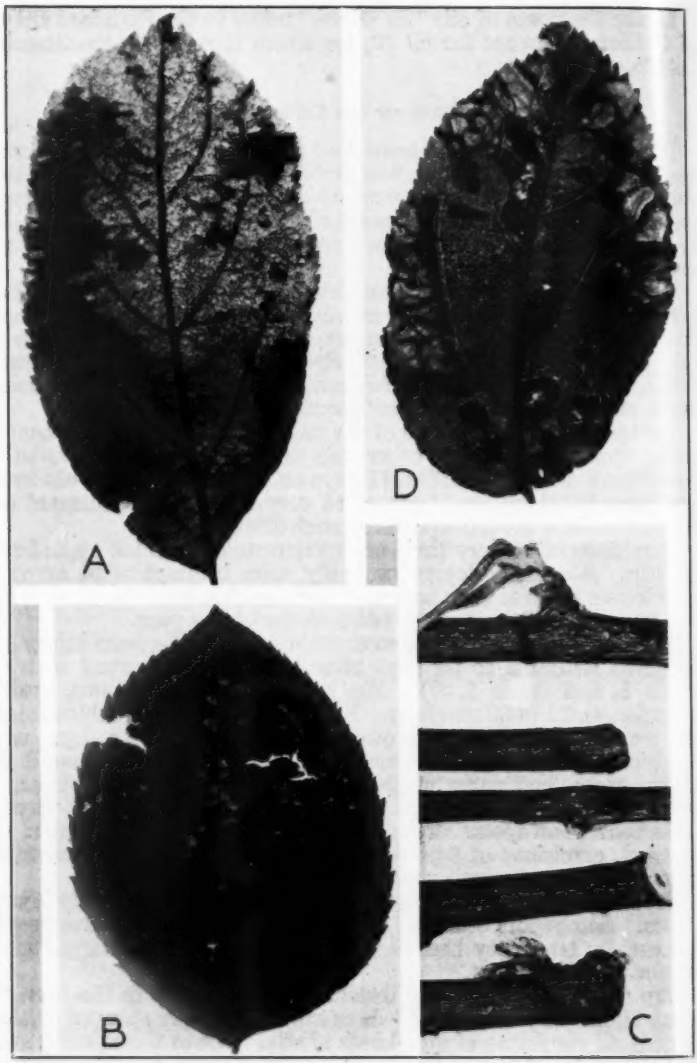

Figure 1.—*A*, Angular mottling in apple leaf 38 days after it was sprayed with a calcium caseinate emulsion of 16-percent oil 24 on July 12, 1929. Gray parts of picture represent the oily, translucent parts of the leaf, while the black regions represent the normal, dark green, nonoily parts of the leaf. × 1. *B*, White and tan epiphyllous necrotic spots in apple leaf 69 days after spraying with a cresoap emulsion of 8-percent oil 21. × 1. *C*, Sharp, raised lines in apple twigs caused by 8- and 16-percent oil emulsions and pure oils applied to their leaves in 1930. The oils passed from the leaves into the twigs and killed the bark beyond the lines. Drying after excision caused the longitudinal wrinkles in the bark. × 1. *D*, Typical brown borders on dry-green leaf spots in a leaf 60 days after spraying with a cresoap emulsion of 8-percent oil 3. × ⅞.

Cresoap emulsions of many oils caused white or light brown, angular or oval, epiphyllous spots 1 to 6 mm wide in apple leaves within 10 to 60 days after spraying (table 1 and fig. 1, *B*). Many of the spots had slightly raised, narrow, purple or dark brown borders. Although most of the spots were only epiphyllous, others were continuous with hypophyllous dark brown spots. Some of the epiphyllous spots had dark central dots (probably physiological). White and tan leaf spots were not seen on unsprayed limbs.

Cresoap emulsions of many oils in 4- and 8-percent concentrations caused white and tan leaf spots in 1930 and 1931, but only oil 13 (31.2 percent sulphonatable) caused them in a concentration of 1-percent oil. Such spots were not seen before 1930, although cresoap emulsions of many of these oils in concentrations of 2 and 4 percent were sprayed on apple leaves in 1929. Perhaps the greater drought injury in this orchard during the last 2 years affected the development of the leaf spots.

All the white and tan spots were caused by cresoap emulsions except some on one limb, which were caused by an ammonia-casein emulsion of 8-percent oil 4 (10 percent sulphonatable).

Delayed dormant sprays of 8- and 16-percent oil emulsions usually killed or severely injured the young leaves which they hit. Oiled parts of juvenile leaves turned light brown and died.

Angular, oval, or irregular epiphyllous, purple or reddish-purple spots 1 to 20 mm wide commonly resulted from sprays with oil 13 (31.2 percent sulphonatable) (table 1). Such spots usually were angular unless they were too small to affect all the epidermis bounded by small veins. The angular spots corresponded in size and shape with angularly mottled spots. Purple spots slowly became necrotic. Epiphyllous and hypophyllous purple spots usually did not occur in the same leaves.

Typical epiphyllous purple spots were caused only by oils 5, 7, 13, 15, 21, and 28, which are 15.6 to 41.6 percent sulphonatable. They were not caused by emulsions of less sulphonatable oils. This leads to the suggestion that the purple spots were caused by certain sulphonatable chemicals sufficiently concentrated only in the six oils listed. Emulsions of other oils with similar sulphonatable residues did not cause purple spots.

Epiphyllous purple spots served as a useful criterion for distinguishing some oils which were too toxic for commercial use on apple leaves. The purple spots were associated with dark brown leaf spots in late stages of development.

Angular, oval, or irregular, hypophyllous spots and sheens were common symptoms of mild injuries by oil emulsions, and these symptoms are included under hypophyllous browning in table 1. These spots and sheens were brown, purple, or silvery, and occurred separately and together in leaves. The epidermis on and between small veins was discolored. In some leaves only the veinal or interveinal epidermis was discolored. The discolorations often occurred in upward-rolled oil-injured leaves and were predominant within 2 cm of the edges. Usually, hypophyllous symptoms had no associated epiphyllous symptoms, but in some severe cases discolored hypophyllous spots were continuous with epiphyllous brown spots.

Chlorosis is a plesionecrotic symptom [7] in which normally green tissues become abnormally light green or yellowish because of decreased amounts of chlorophyll. Instead of causing small chlorotic spots, some oils caused chlorosis of whole leaves or the parts of the leaves between the main veins. Circular mottled spots are included with interveinal and general chlorosis under Chlorosis in table 1.

Small mottled spots often occurred in leaves sprayed with cresoap emulsions of 4- to 16-percent oils. The spots usually were circular, chlorotic, and 1 to 5 mm in diameter. They probable were caused by emulsions entering the leaves, as they were not translucent like the spots caused by free oils.

Oils penetrated abundantly between the cells of leaves and quickly caused translucence of the invaded parts of the mesophyll (fig. 1, *A*, and table 1). Nearly straight veins bounded the oily regions and made them angular. The amounts of oils entering the leaves determined the sizes, number, and degrees of translucence of the angular mottled spots.

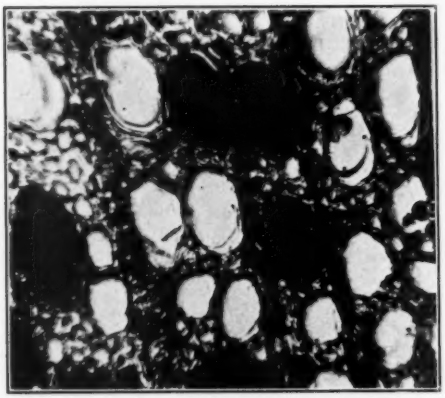

FIGURE 2.—Oil (photographed black) inside trachese of an apple twig 109 days after its leaves were sprayed, with a calcium caseinate emulsion of 16-percent oil 24. × 328.

The oil sign, angular mottling or translucence frequently became a plesionecrotic symptom, angular chlorosis. Many angular mottled spots remained as chlorotic spots after they ceased to be evidently oily translucent.

Angular mottling was a characteristic effect of calcium-caseinate-oil emulsions. One- and two-percent oil-calcium-caseinate emulsions made fewer, smaller, and dimmer translucent spots than did more concentrated emulsions. Translucent spots appeared soon after oil-calcium-caseinate emulsions dried on leaves.

One- to eight-percent oil-ammonia-casein emulsions occasionally caused angularly mottled spots. Probably they penetrated as free oils and also as emulsions so they caused less angular mottling than did the quick-breaking calcium-caseinate-oil emulsions which probably penetrated leaves mainly as free oils.

Cresoap emulsions of 1- to 16-percent oils rarely produced typical angular mottled spots. These stable emulsions presumably penetrated as emulsions. Kelley (*18*) and Ginsburg (*11*) reported penetration of leaves by emulsions.

Emulsions of 8- and 16-percent oils usually deposited on apple leaves and bark obvious films of oil which persisted 1 to 4 weeks after spraying. Such free oil, especially on the hypophyllous hairs of

[7] Plesionecrotic symptoms are those characteristic of the stages of relatively slowly necrotic, abnormal protoplasmic degeneration preceding actual death of cells. Severe chlorosis, slow necrotic spotting, and purpling often are plesionecrotic symptoms.

leaves, held dust, making the affected parts dirty. Emulsions containing 1- to 4-percent oils usually did not make sprayed leaves evidently oily.

Sections of oil-sprayed leaves and their attached twigs revealed oil in and between the cells of many tissues (fig. 2). Abundant internal oils presumably were injurious before necrotic symptoms appeared.

Many oil emulsions caused abnormal degrees of wrinkling, upward rolling, and downward curling of sprayed apple leaves (table 1). Upward rolling of leaf margins often was associated with hypophyllous brown, purple, and silvery sheens.

Leaf dwarfing as a symptom of oil injury to buds is shown in table 2.

TABLE 2.—*Symptoms of injuries most frequently appearing in apple buds as a result of spraying with petroleum oils*

Oil no.	Name of oil	Portion sulphonatable	Viscosity	Oil in spray	Limbs sprayed	Usual degree of injury [a]	Buds killed [a]	Leaves dwarfed [a]	Retarded growth of leaves and flowers [a]
		Percent	*Seconds*	*Percent*	*Number*				
[b]5	Standard 5, no. 14510R	41.6	110	4	76	0	1	1	1
				6	26	1	1	2	2
				8	56	1	1	2	2
				16	59	3	2	3	2
12	Shell Brown Neutral	27	115	4	9	0	1	0	0
				6	9	0	0	0	0
				16	7	2	2	0	0
13	Shell 70 Pale, R.L. 99	31.2	75	4	7	0	0	0	0
				8	12	0	0	2	0
				16	4	2	1	3	1
28	Standard 7, no. 14776R	35.2	100	4	35	0	1	1	1
				6	38	0	1	1	1
				16	39	3	2	2	2
29	Standard (Ind.), Dendrol	30	83	4	14	0	1	0	1
				6	4	0	1	0	0
				16	15	3	3	2	2

[a] The numbers indicate the comparative amount of injury caused by each oil: 0=no symptoms seen; 1=a trace of injury, not commercially serious; 2=injury and symptoms common and serious; 3=symptoms abundant and destructive, causing severe injury.

[b] Includes sprays on opening buds on May 14, 1929.

Oil emulsions were sprayed on apple leaves 1 to 2 cm long in the orchard during blooming, on May 26, 1930. Four-percent oil emulsions of the oils noticeably dwarfed the leaves on some branches. One-percent oil emulsions did not definitely dwarf the leaves. Emulsions of 8- and 16-percent oils usually decreased or prevented subsequent growth of sprayed leaves. Leaf dwarfing was associated with necrosis of the leaves and flowers in many cases. These observations showed that 4- to 16-percent oil emulsions were commercially objectionable on juvenile leaves.

SYMPTOMS IN BUDS AND BARK

The opening and later development of buds was delayed by 16-percent oil emulsions sprayed on dormant buds (table 2). Opening buds were damaged by sprays of 8-percent oil emulsions. Latent buds developed on some limbs on which oils had killed the dominant buds. Emulsions of 4-percent oils rarely caused these symptoms.

Lethal quantities of oils entered some sprayed buds, causing them to become brown and dry. Lethal quantities of oils also passed from sprayed leaves into some of the attached twigs, causing the buds to die and the bark to become dry, brown, and shriveled. The rapidity and extent of the killing was generally directly proportional to the sulphonatable residue and concentration of each oil applied.

Some 8- and 16-percent oil emulsions killed the twigs attached to sprayed leaves. Such twigs typically developed sharp, narrow lines separating living and dead bark (fig. 1, *C*).

Symptoms in Flowers

Apple trees were sprayed on May 26, 1930, when in bloom. Most of the 4-, 8-, and 16-percent oil emulsions killed the sprayed flowers, turning the petals brown within a few days. Many oil-killed flowers remained attached to their twigs for a few weeks. Comparable 1-percent oil emulsions did not kill sprayed flowers.

Symptoms in Fruit

Red-bordered black spots 1 to 3 cm wide occurred in the sunny sides of maturing apples on two limbs after spraying with emulsions of 8-percent oil 21 (15.6 percent sulphonatable), and 4-percent oil 4 (10 percent sulphonatable).

Russeted and cracked apples occurred on a limb sprayed with 4-percent oil 22 (2 percent sulphonatable). Abnormal fruit color was associated with oil sprays on many limbs in 1928. One- and two-percent oil sprays did not cause these symptoms.

Oil Injuries to Apple Buds

Oil emulsions were sprayed on apple buds and limbs in February, March, and April from 1928 to 1931, to determine which emulsions were safe for use on dormant buds.

No differences in effects of emulsions were found that could be attributed to differences in the emulsifiers.

Emulsions in concentrations of 4-percent oil rarely caused serious symptoms of injuries in sprayed buds, even after delayed-dormant sprays (table 2).

Emulsions of 6- and 8-percent oils frequently caused no symptoms in sprayed buds, especially in buds sprayed in February and March. However, many buds sprayed with 6- and 8-percent oils were seriously injured, especially following sprays on opening buds (table 2).

Emulsions of 16-percent oils usually were injurious. They were used only to reveal and make distinctly recognizable the symptoms of oil injuries.

QUALITIES OF EMULSIFIERS

Emulsifiers differ in their toxic effects and the ways in which they alter the miscibilities of emulsified-oil droplets with plant liquids. Emulsifiers probably facilitate the spread of oil in plants, and the mixing of oil in plant liquids. Emulsions may have the combined toxic effects of the oils and emulsifiers.

Cresoap

Two percent of cresoap in water caused severe browning in apple leaves within 1 day and caused partial defoliation within a month. Concentrations of one fourth of 1 percent and of 1 percent of cresoap

in water caused no symptoms in sprayed leaves. A cresoap emulsion of 8-percent petroleum oil contains 0.89 percent of cresoap; this concentration of cresoap alone is probably not injurious. Concentrations of 1.78 percent of cresoap evidently were seriously toxic when they occurred in 16-percent oil emulsions of oil 3 (5 percent sulphonatable) and oil 24 (unsulphonatable) because these emulsions killed sprayed apple leaves within 1 to 30 days, while these oils in undiluted form and also in stock emulsions made with calcium caseinate and ammonia casein were much less injurious in comparable tests.

No deterioration of cresoap stock miscible oils was observed during 2 years, and dilution emulsions were made easily and accurately from them. Oil droplets 1μ to 5μ in diameter or smaller predominated in the emulsions. Four-percent cresoap-oil emulsions were stable for more than 16 months.

Stock emulsions of Solar Red Engine oil (Standard Oil Co.) and Diamond Paraffin oil were made in Illinois in 1923, according to the formula: 500 cc of oil, 125 cc of water, and 127.6 g of potassium-fish-oil soap emulsified by boiling and stirring with an electric stirrer. Dilutions of these stock emulsions resembled in stability and macroscopic appearance the cresoap emulsions made in 1927 to 1932.

Calcium-Caseinate Emulsifiers

Calcium-caseinate-oil emulsions were unsatisfactory because they creamed and broke very rapidly, and apparatus was not available to provide sufficient agitation and pressure to secure uniform applications of oil. Sprayed leaves on three limbs were not injured by a suspension of 1 percent of calcium caseinate in water. A 1-percent oil-calcium-caseinate emulsion contains only 0.022 percent of calcium caseinate, so this emulsifier is not an important factor in the toxicity of calcium-caseinate-oil emulsions.

Ammonia-Casein Emulsifiers

Sprayed leaves on three limbs for each concentration were not injured by the ammonia-casein emulsifier in water in the concentrations in which it occurs in 1-, 4-, 8-, and 16-percent oil-ammonia-casein emulsions. These concentrations of ammonia casein ranged from 0.016 to 0.256 percent, and were not considered to be important factors in the toxic effects of ammonia-casein-oil emulsions. Ammonia-casein emulsifiers made rapid creaming emulsions, but they were stable enough to permit uniform applications of oils.

Semisolid Stock Emulsions

The semisolid stock emulsions of oils 23 and 30 were difficult to dilute accurately. Oil 23 was broken when sunlight warmed the containing can to about 50° C.

CONDITIONS AFFECTING OIL INJURIES

Correlated Injuries Caused by Oils and Drought

Nearly half of the oil-sprayed apple trees showed leaf symptoms of drought injury each summer. Observations in 1929 and 1930 revealed that the leaves on 102 limbs showed greater oil injury than corresponding oil-sprayed leaves on trees not showing symptoms of drought injury. No correlation was observed on 46 other limbs, be-

cause drought injury caused no apparent increase in oil injury. Thus, most of the evidence showed a direct correlation between injuries caused by drought and by 4-, 8-, and 16-percent oil emulsions of 12 kinds of oils sprayed on apple leaves. Observations in 1928 and 1931 also supported the conclusion that it is more dangerous to use oils on Hibernal apple leaves evidently suffering from drought than on normal leaves.

Water in and between cells theoretically constitutes the main barrier to the spread of petroleum oils in leaves, so oils are expected to spread less in normal leaves than in leaves suffering from drought.

Temperature, Humidity, and Wind

Daily records of temperature, humidity, precipitation, hours of sunshine, and velocity and direction of wind were kept at an official Weather Bureau station of the United States Department of Agriculture located nearly 400 meters southeast of the apple orchard. The records for the days when sprays were applied are not included because there was no correlation between the weather and the effects of the sprays.

Severe injuries were caused occasionally by 1- and 2-percent oil emulsions, perhaps affected by unusual conditions in the trees and weather. Oils more than 10 percent sulphonatable have small margins of safety even in 1- and 2-percent concentrations and may cause severe injuries. Unusual weather conditions occur frequently. Probability of occasional serious injury is a recognized hazard in commercial spraying.

Sprays froze on the trees on three dates, but the freezing made no apparent difference in the effects of the sprays. Unpublished data show that freezing usually did not break 4- and 8-percent oil-cresoap emulsions. The unstable calcium-caseinate emulsions broke and creamed so quickly that freezing probably was not important.

Other Injuries

Wounds and dead spots in leaves and injuries made by mites, leaf hoppers, and aphids presumably facilitate penetration of oils, so enlarged wounds were a common symptom of oil injury.

In many cases, shaded leaves appeared to be injured less by oils than comparably sprayed leaves in sunlight.

The Hibernal apple orchard showed no evidence of cumulative injury by oil sprays during 6 years. The injuries caused by oil sprays were associated with individual oils applied on known dates.

DISCUSSION

Oil injuries in Hibernal apple leaves generally were correlated with the percentages of sulphonatable residues in the oils. Oils with high percentages of sulphonatable residues, in comparison with oils having low percentages of sulphonatable residues, usually caused a more rapid appearance of severe symptoms.

Exact correlations between sulphonatable residues and injuries were not expected, because sulphonatable chemicals presumably differ in their compositions and toxic effects. Thus, oils 16, 20, and 24 were more injurious to leaves than their sulphonatable residues of 10, 2, and 0 percent indicated.

The behaviors of such oils may be explained partly because some unsaturated compounds in oils are also unsulphonatable, so that even an oil showing no sulphonatable residue probably contained some unsaturated compounds. For example, some heavy olefines and parts of the naphthenic acids and sulphur compounds are not removed by sulphuric acid (Swingle and Snapp (*34*)), and some heavy naphthenes are not removed by sulphuric acid.

Eleven of the oils described in table 1 were tested by Green (*14*) who found that apple leaves and barley seedlings were similarly affected; they were most injured by the oils which were more than 5 percent sulphonatable. He determined the sulphonatable residues in these 11 oils and found close correlation between sulphonatable residues and injuries.

Green and Johnson (*13*) determined that three of these oils that were less than 16 percent sulphonatable decreased respiration of bean leaves, and three of them that were more than 16 percent sulphonatable increased respiration.

Oils have two main effects on leaves: (1) They plug stomata, intercellular spaces, and tracheae; and (2) the toxic parts of oils poison protoplasm. Apparently the toxic effects exceeded the physical effects of the oils more than 16 percent sulphonatable in the experiments cited in the preceding paragraph. Thus, oils with sufficiently concentrated sulphonatable chemicals had the predominating effect of increasing respiration in acting like other toxic chemicals as described by Kostychev and Lyon (*20*).

In these experiments there was no apparent correlation between degree of leaf injuries and differences in viscosities of spray oils varying between 50 and 108 seconds. However, Knight, Chamberlin, and Samuels (*19*) reported that leaves recovered more quickly from oils with low viscosities than from oils with high viscosities. Spuler, Overley, and Green (*33*) said that six applications of spray oils with viscosities of 70 to 120 seconds on heavily loaded trees reduced the sizes of apples. One application of oil injured Yellow Newtown apples.

Viscosities, volatilities, and distillation ranges are related qualities which are reported in literature as affecting the physiological effects of oils on organisms. Fairly straight-cut petroleum oils behave more uniformly in these qualities, so they are preferable to blended oils in sprays.

The viscosity of an oil is correlated with its rate of penetration and is determined at 37.7° C., a temperature which occurs in orchards, so viscosity probably is more significant than volatility or distillation range.

The relationship between volatilities determined at 50° to 100° C., distillation ranges determined at 288° to 399° (*30*), and volatilities of oils in orchards at 20° to 40° appears to be important and needs further investigation.

Nacent oxygen from photosynthesis in leaves may increase the oxidation of oils on and in leaves, forming oxides and peroxides. Thin films of oils exposed to air and sunlight may decompose and form acids injurious to leaves.

The chemical activity of an oil is increased, and its viscosity is decreased, by increasing its temperature. The speed with which an oil penetrates is increased by decreasing its viscosity. Hence, an oil at

25° C. is more active chemically and penetrates more rapidly than the same oil at 0°.

Agitation of sprayed leaves increases the amount of emulsion running off, so it decreases the amount of oil remaining and penetrating. The relation between the rates of evaporation and breaking of an emulsion determines the amounts of oil entering leaves in emulsified and unemulsified forms. Evaporation of water from emulsions on leaves is increased by increasing wind velocities and decreasing humidities.

Oil might delay or reduce the development of dormancy in predormant apple trees, and thus increase the hazard of winter injury. Hence, trees were not sprayed in October, November, and December.

SUMMARY AND CONCLUSIONS

The effects of petroleum-oil emulsions on apple were tested by spraying 25 kinds of oils on the leaves of 2,566 apple limbs during 7 years.

Oil sprays caused the following main symptoms in apple leaves: Premature defoliation; large brown spots; epiphyllous purple spots; hypophyllous brown, purple, and silvery spots; and chlorotic, translucent spots.

Epiphyllous purple spots served to distinguish some oils that were too toxic for commercial use on apple leaves.

The experiments showed that oils for applications to Hibernal apple leaves should not be more than 15 percent sulphonatable, and that oils only 5 to 10 percent sulphonatable were preferable. Oil injuries to apple leaves and buds usually increased with increasing amounts of sulphonatable residues in the oils. The different sulphonatable chemicals in the oils appeared to differ in their effects on apple. This probably explains why low concentrations of some oils were more injurious than higher concentrations of some other oils.

Oils usually were more injurious to drought-injured leaves than to normal leaves.

On the basis of injury to apple, the cresoap emulsifier was preferable to calcium caseinate, because cresoap emulsion of 1- to 8-percent oils were more easily diluted, more uniformly applied, and were less injurious to leaves.

The experiments showed that oils for application to Hibernal apple buds should not be more than 45 percent sulphonatable, and that oils only 30 to 40 percent sulphonatable were preferable.

Before using in orchards, each oil should be compared in concentrations of 16 and 100 percent with an unsulphonatable, similarly viscous oil on mature apple leaves to secure evidence of oil symptoms. Oils killing large parts of leaves within a week probably are too injurious for use on apple leaves. One- and two-percent oil emulsions which cause visible symptoms other than slight angular mottling in apple leaves are too injurious for commercial use on leaves. The speed with which an oil kills cells or causes plesionecrotic symptoms is an important index of its toxicity as a spray oil.

LITERATURE CITED

(1) Anderson, H. W., Flint, W. P., Farrar, M. D., and Smith, M. A.
1930. spray coverage. Jour. Econ. Ent. 23: 466–469.

(2) Bartholomew, E. T.
1929. penetration of spray oil into citrus tree tissues. Calif. Citrogr. 14: 337.

(3) Browne, A. C.
1930. The oil emulsions, a brief survey. Calif. Dept. Agr. Bul. 19: 389–408, illus.
(3a) Burroughs, A. M.
1923. A new method of making engine oil emulsions. Missouri Agr. Expt. Sta. Bul. 205, 8 p., illus.
(4) Clements, F. E.
1909. The genera of fungi. 227 p. Minneapolis, Minn.
(5) Ebeling, W.
1932. Experiments with oil sprays used in the control of the California red scale, Chrysomphalus aurantii (Mask.) (Homoptera: Coccidae) on lemons. Jour. Econ. Ent. 25: 1007–1012, illus.
(6) Farrar, M. D.
1930. Effect of oil sprays on orchard fruit and foliage. Ind. Hort. Soc. Trans. 70: 76–80.
(7) Felt, E. P.
1913. Injuries following the application of petroleum oils or petroleum products to dormant trees. Jour. Econ. Ent. 6: 160–161.
(8) Ginsburg, J. M.
1929. A correlation between oil sprays and chlorophyll content of foliage. Jour. Econ. Ent. 22: 360–366.
(9) ———
1929. Summer oil sprays and their effect on apple trees. N.J. Agr. Expt. Sta. Rpt. 50: 173–176.
(10) ———
1930. Studies on penetration of oils into plant tissue. N.J. Agr. Sta. Rpt. 51: 163–167.
(11) ———
1931. Penetration of petroleum oil into plant tissue. Jour. Agr. Research 43: 469–474.
(12) ———
1931. What summer oils may do to apple trees. Jour. Econ. Ent. 24: 283–290, illus.
(13) Green, J., and Johnson, A. H.
1931. The effect of petroleum oils on the respiration of bean leaves. Plant Physiol. 6: 149–159, illus.
(14) Green, J. R.
1932. Chemical and physical properties of petroleum spray oils. Jour. Agr. Research 44: 773–787, illus.
(15) Hartzell, F. Z.
1931. Investigations aimed at reducing the cost of pear psylla control. Jour. Econ. Ent. 24: 71–77.
(16) Haynes, R.
1930. Detecting oil in twigs. Stain Technol. 5: 29.
(17) Kelley, V. W.
1930. Effect of certain hydrocarbon oils on respiration of foliage and dormant twigs of the apple. Ill. Agr. Expt. Sta. Bul. 348, p. 371–406, illus.
(18) ———
1930. Effect of certain hydrocarbon oils on the transpiration rate of some deciduous tree fruits. Ill. Agr. Expt. Sta. Bul. 353, p. 581–600.
(19) Knight, H., Chamberlin, J. C., and Samuels, C. D.
1929. On some limiting factors in the use of saturated petroleum oils as insecticides. Plant Physiol. 4: 299–321, illus.
(20) Kostychev, S., and Lyon, C. J.
1927. Plant respiration. Transl. and ed. by C. J. Lyon. 163 p., illus. Philadelphia.
(20a) Melander, A L., Spuler, A. and Green, E. L.
1924. Oil sprays; their preparation and use for insect control. Wash. Agr. Exp. Sta. Bul. 184, 31 p.
(21) Newcomer, E. J., and Yothers, M. A.
1931. Experiments for the control of the San Jose scale with lubricating-oil emulsions in the Pacific Northwest. U.S. Dept. Agr. Circ. 175, 12 p., illus.

(22) NEWCOMER, E. J., and YOTHERS, M. A.
1932. EXPERIMENTS WITH INSECTICIDES FOR CODLING MOTH CONTROL. U.S. Dept. Agr. Tech. Bul. 281, 29 p., illus.
(23) OVERHOLSER, E. L., and OVERLEY, F. L.
1930. EFFECT OF OIL SPRAYS ON APPLE TREES. Wash. Agr. Expt. Sta. Sta. Bul. 245: 43.
(24) PENNY, D. D.
1932. THE EFFECTS OF OIL SPRAY ON THE NAVEL ORANGE. Jour. Econ. Ent. 25: 1002–1007.
(25) PROEBSTING, E. L.
1925. THE RELATION OF STORED FOOD TO CAMBIAL ACTIVITY IN THE APPLE. Hilgardia 1: [81]–106, illus.
(26) PROESCHER, F.
1927. OIL RED O A RAPID FAT STAIN. Stain Technol. 2: 60.
(27) QUAINTANCE, A. L., NEWCOMER, E. J., and PORTER, B. A.
1931. LUBRICATING-OIL SPRAYS FOR USE ON DORMANT FRUIT TREES. U.S. Dept. Agr. Farmers' Bul. 1676, 18 p., illus.
(28) ROBINSON, R. H., FISHER, D. F., and SPULER, A.
1930. THE WESTERN COOPERATIVE OIL SPRAY PROJECT. Science (n.s.) 71: 440–441.
(29) SMITH, R. H.
1929. STUDIES ON SPRAY TANK AGITATION IN THE USE OF OIL SPRAYS. Jour. Econ. Ent. 22: 929–934.
(30) ———
1932. THE TANK-MIXTURE METHOD OF USING OIL SPRAY. Calif. Agr. Expt. Sta. Bul. 527, 86 p., illus.
(31) SPULER, A.
1930. OIL SPRAYS. Wash. Agr. Expt. Sta. Bul. 245: 31–33.
(32) ——— OVERLEY, F. L., and GREEN, E. L.
1931. OIL SPRAYS FOR DORMANT USE. Wash. Agr. Expt. Sta. Bul. 247, 27 p., illus.
(33) ——— OVERLEY, F. L., and GREEN, E. L.
1931. OIL SPRAYS FOR SUMMER USE. Wash. Agr. Expt. Sta. Bul. 252, 39 p., illus.
(34) SWINGLE, H. S., and SNAPP, O. I.
1931. PETROLEUM OILS AND OIL EMULSIONS AS INSECTICIDES, AND THEIR USE AGAINST THE SAN JOSE SCALE ON PEACH TREES IN THE SOUTH. U.S. Dept. Agr. Tech. Bul. 253, 48 p., illus.
(35) WOODHAMS, G. E.
1931. PETROLEUM OIL SPRAYS ON NURSERY STOCK. Calif. Dept. Agr. Mo. Bul. 20: 325–330.
(36) WOODWORTH, C. W.
1930. PETROLEUM INSECTICIDES. Jour. Econ. Ent. 23: 848–851.
(37) YOUNG, P. A.
1930. SPRAY-OIL INJURY IN APPLE LEAVES AND LIMBS. (Abstract) Phytopathology 20: 122.
(38) ———
1931. PENETRATION AND TOXICITIES OF PETROLEUM-OIL SPRAYS. (Abstract) Phytopathology 21: 130.

SELF- AND CROSS-POLLINATION STUDIES OF APPLE VARIETIES, PARTICULARLY ROME BEAUTY AND GALLIA BEAUTY [1]

By FREEMAN S. HOWLETT

Associate horticulturist, Ohio Agricultural Experiment Station

INTRODUCTION

During the last decade several apple varieties of the Rome Beauty type, which show considerable improvement in color of fruit, have been receiving favorable attention. Gallia Beauty, reported by Gourley and Ellenwood (*7*)[2] to be a seedling of Rome Beauty, is now being planted in a number of fruit-growing districts, of which Ohio is a typical example.

The question has thus arisen as to the degree of self-fruitfulness of Gallia Beauty and its interfruitfulness with other varieties of the Rome Beauty type. In 1927 the writer (*9*) presented data indicating that Gallia Beauty was not an effective pollinizer of Rome Beauty. At that time only a few flowers were used in the experiments. In 1930 further preliminary results were published (*12*) substantiating the previous conclusion. It was also concluded that Gallia Beauty, like Rome Beauty, possesses the characteristic of self-fruitfulness to a greater extent than do other varieties studied at the Ohio Agricultural Experiment Station. The amount of fruit produced from selfing, however, was not sufficiently large to give full crops of fruit. However, in 1930, Knowlton (*13*) published data which seemed to indicate that Gallia Beauty was an effective pollinizer of Rome Beauty. But very recently (*14*) he presented further data confirming the conclusions here reached.

The primary purpose of the experiments reported herein was (1) to obtain more extensive data regarding the self-fruitfulness and interfruitfulness of Rome Beauty and Gallia Beauty, (2) to determine the extent of possible seasonal variation in the self-fruitfulness of Gallia Beauty, Rome Beauty, Baldwin, and Jonathan, and (3) to determine the comparative value, as pollinizers, of several varieties whose planting in commercial orchards is relatively recent.

EXPERIMENTAL PROCEDURE

Selected branches of vigorous trees were inclosed in large cheesecloth bags, just as the terminal flowers of the clusters were about to open. The methods used were practically the same as those described in a previous publication (*12*). In the selfing experiments usually the entire cluster was hand-pollinated. In the cross-pollination work two large lateral flowers were usually pollinated, all other flowers being removed. Emasculation, when practiced (as shown in tables

[1] Received for publication Aug. 26, 1932; issued October, 1933.

[2] Reference is made by number (italic) to Literature Cited, p. 536.

1, 2, and 4), consisted only of a removal of petals and stamens. When the flowers were not emasculated, pollination was carefully done by means of a camel's-hair brush.

PRESENTATION OF THE DATA

The data from the self-, cross-, and open-pollinated flowers are presented on the basis of the percentage of flowers that set fruit. The set after both the first and second (June) drop is given in tables 1, 2, and 4. In these tables is also given the percentage of set of the open-pollinated branches after the excess fruits had been removed. In Rome Beauty and Gallia Beauty this thinning, as indicated in table 5, consisted almost entirely of the reduction of the number of fruits per cluster to one. In Baldwin, Delicious, Jonathan, and Turley, the clusters were also thinned to one fruit per spur, and in addition the fruiting points were spaced so that the minimum distance between adjacent fruits was 8 inches. The open-pollinated branches had usually set abundantly. Except as otherwise indicated in the tables, the fruits left on the open-pollinated branches after thinning represented, in the writer's judgment, the maximum which the branches should hold to maturity. This number expressed in percentage of the original number of flowers on the branch is termed a "full crop" for the particular tree concerned and can be readily compared with the percentage of flowers setting fruit, on the hand-pollinated branches.

SELF-POLLINATION OF ROME BEAUTY

The data presented in table 1 show that an appreciable number of fruits were obtained from selfing Rome Beauty. In 1930 one of two selfed branches gave 0.8 percent. In 1931 the set ranged from 1 to 5.2 percent on 6 branches of 4 trees; in 1932 on a similar number of branches of 2 trees, the set ranged from 0 to 1.4 percent. Usually the higher percentages were obtained from partly deflorated clusters.

TABLE 1.—*Results of self- and cross-pollination of Rome Beauty, 1930–32*

TREE 430, 1930

Pollen parent	Treatment of flowers in cluster	Number of flowers	Percentage of flowers setting fruit—		
			After first drop	After second drop	After thinning
Rome Beauty	None emasculated	370	1.4	0.8	
	do	100	2.0	0	
Gallia Beauty	2 not emasculated	60	3.3	0	
	do	52	0	0	
Jonathan	None emasculated	173	1.7	.6	
	do	122	44.3	24.6	
Open pollinated		695	49.3	32.7	9.6

TREE 324, 1931

Rome Beauty	2 not emasculated	136	13.3	5.1	
Gallia Beauty	do	118	.9	0	
Golden Delicious	do	330	21.2	11.6	
Open pollinated		1,127	49.2	13.2	9.5

TABLE 1.—*Results of self- and cross-pollination of Rome Beauty, 1930-32*—Contd.

Pollen parent	Treatment of flowers in cluster	Number of flowers	Percentage of flowers setting fruit— After first drop	After second drop	After thinning
TREE 325, 1931					
Rome Beauty	2 not emasculated	134	23.1	2.2	
Gallia Beauty	do	154	4.5	0	
	do	156	0	0	
Golden Delicious	do	178	2.8	2.8	
Open pollinated		1,001	51.8	10.5	8.3
TREE 326, 1931					
Rome Beauty	2 not emasculated	211	7.6	5.2	
Gallia Beauty	do	119	1.7	.9	
	do	135	5.9	1.5	
Melba	do	108	14.8	13	
Open pollinated		1,110	44.4	10.4	8.1
TREE 430, 1931					
Rome Beauty	None emasculated	306	1.0	1.0	
	do	102	1	1	
	2 not emasculated	129	5.4	5.4	
Gallia Beauty	do	33	0	0	
	do	131	5.4	5.4	
	2 emasculated	81	1.2	0	
	do	99	4.2	4.2	
	do	156	.6	.6	
Rome Beauty, No. 73	do	105	0	0	
	do	114	2.6	2.6	
	do	186	0	0	
Jonathan	do	103	18.5	18.5	
Starking	do	128	6.3	6.3	
Open pollinated		1,172	62.8	20.2	12.5
TREE 326, 1932					
Rome Beauty	None emasculated	140	1.4	1.4	
	2 emasculated	20	0	0	
	do	72	1.4	1.4	
Gallia Beauty	do	75	2.6	2.6	
	do	65	0	0	
	do	39	0	0	
Red Spy	do	59	23.7	23.7	
Golden Delicious	do	39	46.1	36	
Open pollinated		660	24.3	21.6	11.7
TREE 430, 1932					
Rome Beauty	None emasculated	277	1.1	.7	
	do	415	.7	0	
Gallia Beauty	2 emasculated	102	11.7	8.8	
	2 not emasculated	112	2.7	2.7	
	2 emasculated	108	0	0	
Rome Beauty No. 73	do	69	0	0	
	do	108	0	0	
Golden Delicious	2 not emasculated	64	54.7	46.8	
	2 emasculated	65	7.7	7.7	
Open pollinated		1,035	30.9	28.9	16.8

In no case was the number of fruits produced equivalent to a full crop. In 1930 the set of fruit on the open-pollinated branches after thinning was 9.6 percent; in 1931 the set on the open-pollinated branches ranged from 8.1 to 12.5 percent and in 1932 from 11.7 to

16.9 percent. If the degree of self-fruitfullness of the variety is expressed as the relation between the respective percentages of the selfed and open-pollinated branches, in 1930 the selfed limb produced 8 percent of a full crop. In 1931 trees 324, 325, and 326 gave 53, 26, and 64 percent, respectively, of a full crop. In 1932 the two trees used in the experiment produced 12 and 52 percent of a full crop, respectively.

SELF-POLLINATION OF GALLIA BEAUTY

The data presented in table 2 show that small but significant percentages of set were also obtained from selfing Gallia Beauty. On the one tree used in 1930 the set on three branches was 2.7, 5.7, and 6.3 percent; in 1931 the set in three branches of a companion tree was 0, 1.2, and 4.9 percent. In this case the higher percentages were obtained from partially deflorated clusters. In 1932 the set on seven selfed limbs of one tree ranged from 0 to 3.4 percent, while on another tree no fruit was obtained from selfing.

TABLE 2.—*Results of self- and cross-pollination of Gallia Beauty, 1930–32*

Pollen parent	Treatment of flowers in cluster	Number of flowers	Percentage of flowers setting fruit—		
			After first drop	After second drop	After thinning
TREE 16, 1930					
Gallia Beauty	None emasculated	192	10.9	5.7	
	do	186	5.4	2.7	
	do	127	8.7	6.3	
Rome Beauty	2 not emasculated	97	3.1	3.1	
	do	77	1.3	0	
	2 emasculated	99	8.1	6.1	
McIntosh	None emasculated	148	3.4	3.4	
	2 emasculated	58	39.7	22.4	
	do	84	39.3	28.6	
Open pollinated		355	67.6	23.9	14.9
TREE 4, 1931					
Gallia Beauty	None emasculated	255	0	0	
	2 not emasculated	183	7.1	1.2	
	do	153	13.1	4.9	
Rome Beauty	2 emasculated	160	10.6	3.1	
	do	92	2.2	1.1	
	do	114	.9	.9	
Stark's Dark Red Rome	do	136	3.7	3.0	
	do	144	.7	.7	
Rome Beauty No. 73	do	106	11.3	5.0	
	do	60	8.3	1.7	
Golden Delicious	do	173	4.6	1.7	
Jonathan	do	218	12.0	6.9	
Starking	do	103	20.4	16.5	
Open pollinated, branch 1		117	40.2	30.9	
Open pollinated, branch 2		459	73.8	16.7	8.7
		354	69.5	16.7	9.3
TREE 16, 1932					
Gallia Beauty	None emasculated	498	4.4	1.6	
	do	494	9.1	3.4	
	2 emasculated	87	0	0	
	do	234	1.0	.4	
	do	175	2.1	.6	
	do	187	0	0	
	do	96	0	0	

TABLE 2.—*Result of self- and cross-pollination of Gallia Beauty, 1930–32*—Contd.

TREE 16, 1932—Continued

Pollen parent	Treatment of flowers in cluster	Number of flowers	Percentage of flowers setting fruit—		
			After first drop	After second drop	After thinning
Rome Beauty	2 emasculated	171	0	0	
	do	142	.7	0	
	do	209	0	0	
	do	128	0	0	
	do	93	14.0	6.5	
Rome Beauty No. 73	do	122	0	0	
	do	213	1.0	0	
Golden Delicious	do	160	.6	.6	
McIntosh	do	122	52.3	24.8	
Red Spy	do	95	49.4	29.4	
	do	107	49.5	19.6	
Open pollinated	do	138	72.4	49.3	
		1,157		17.5	9.3

TREE 4, 1932

Gallia Beauty	2 emasculated	171	0	0	
	do	43	0	0	
Rome Beauty	do	63	0	0	
	do	77	5.2	1.3	
Red Spy	do	79	0	0	
	do	37	91.9	89.2	

The fruits produced by selfing were in no case equivalent to a full crop. In 1930 the set on the three selfed limbs was equivalent to 18, 38, and 42 percent of a full crop. In 1931 the maximum percent from selfing was equivalent to 54 percent, and in 1932 to 36 percent, of a full crop.

CROSS-POLLINATION OF ROME BEAUTY

The principal pollination work with Rome Beauty was designed to test the effectiveness of Gallia Beauty as its pollinizer. A variety supposedly a seedling of Rome Beauty, and known as "Rome Beauty No. 73", was also used as a pollinizer. This variety is almost identical in color to Gallia Beauty but blooms a day or two later.

With but one exception (one branch on tree 326 in 1932), no higher set was obtained from cross-pollinating Rome Beauty with Gallia Beauty than from selfing Rome Beauty. It is obvious that on those branches where the flowers were not emasculated some of the fruits produced might have been from selfed flowers. Where the flowers were emasculated, Gallia Beauty gave, in several instances, percentages as high as were obtained from selfing Rome Beauty. Rome Beauty No. 73 also produced similar results. On the other hand, Golden Delicious, Jonathan, Melba, Starking, and Red Spy were very effective pollinizers.

In view of the difference in the set of fruit produced by Starking and Jonathan as pollinizers of Rome Beauty tree 430 in 1931, it might be assumed that these varieties differ in their effectiveness as pollinizers of Rome Beauty. The writer is of the opinion that other data than the percentage of flowers setting fruit must also be considered before such conclusions can be drawn. On this point, Crane and Lawrence (*3*) have suggested that the germination capacity of the seeds should

be taken as an index of the value of good pollinizers. The writer is of the opinion that both seed number per fruit (empty and plump seeds to be separated) and the germination capacity of the seeds are necessary before one should conclude that certain good varieties differ significantly in their effectiveness as pollinizers. The number of seeds in the fruits obtained from the self- and cross-pollination experiments is given in table 3. There was obviously no significant difference between the number of seed in the fruits produced by Jonathan and Starking as pollinizers of Rome Beauty. On the other hand the number of seeds in the selfed fruits of Rome Beauty, as well as in those produced by Gallia Beauty as the pollinizer, ranged from three to four per fruit.

TABLE 3.—*Number of well-filled and empty or flattened seeds of self- and cross-pollinated fruits of different apple varieties, 1930 and 1931*

Pollen parent	Total fruits	Seeds per fruit—		
		Well-filled	Empty or flattened	Total
ROME BEAUTY, TREE 324, 1931				
Rome Beauty	7	3.3	0.1	3.4
Golden Delicious	19	6.2	1.0	7.2
ROME BEAUTY, TREE 325, 1931				
Rome Beauty	3	3.0	1.0	4.0
Golden Delicious	5	5.8	.6	6.4
ROME BEAUTY, TREE 326, 1931				
Rome Beauty	4	4.0	0	4.0
Melba	14	5.3	.7	6.0
ROME BEAUTY, TREE 430, 1931				
Rome Beauty	10	3.4	0.1	3.5
Gallia Beauty	11	2.6	.4	3.0
Rome Beauty No. 73	3	4.0	0	4.0
Jonathan	17	6.0	.1	6.1
Starking	7	6.3	.3	6.6
GALLIA BEAUTY, TREE 16, 1930				
Gallia Beauty	24	4.9	.8	5.7
Rome Beauty	10	4.5	0	4.5
McIntosh	28	5.3	1.0	6.3
Open pollinated	136	7.0	.2	7.2
GALLIA BEAUTY, TREE 4, 1931				
Gallia Beauty	9	3.2	0.3	3.5
Rome Beauty	12	2.8	.8	3.6
Stark's Dark Red Rome	5	3.6	.2	3.8
Rome Beauty No. 73	2	1.5	.5	2.0
Golden Delicious	15	5.9	.7	6.6
Jonathan	14	7.4	.5	7.9
Starking	36	6.9	.3	7.2

TABLE 3.—*Number of well-filled and empty or flattened seeds of self- and cross-pollinated fruits of different apple varieties, 1930 and 1931*—Continued

Pollen parent	Total fruits	Seeds per fruit—		
		Well-filled	Empty or flattened	Total
DELICIOUS, TREE 279, 1931				
Cortland	50	6.4	0.9	7.
Gallia Beauty	82	7.0	.3	7.3
Golden Delicious	24	6.4	.1	6.5
Jonathan	48	6.2	.2	6.4
JONATHAN, TREE 445, 1931				
Jonathan	29	2.7	0	2.7
Starking	47	3.9	.2	4.1
TURLEY, TREE 477, 1931				
Turley	1	1.0	1.0	2.0
Delicious	10	3.4	2.7	6.1
Jonathan	2	5.0	1.5	6.5

CROSS-POLLINATION OF GALLIA BEAUTY

One phase of the experiments was largely designed to test the effectiveness of Rome Beauty as a pollinizer of Gallia Beauty. Rome Beauty No. 73 and Stark's Dark Red Rome were also used as pollinizers.

The data presented in table 2 show that Rome Beauty, with the exception of 1 limb (tree 16 in 1932) never produced more fruit as a pollinizer of Gallia Beauty than Gallia Beauty gave on selfing. Rome Beauty in several instances produced as high sets on Gallia Beauty as did Gallia Beauty when selfed. Stark's Dark Red Rome and Rome Beauty No. 73 gave similar results. On the other hand, McIntosh, Delicious, Jonathan, Starking, and Red Spy were very effective pollinizers.

The number of seeds (table 3) in the fruits produced by the good pollinizing varieties was not significantly different. The seed content of the fruits produced by selfing and by crossing with Rome Beauty, Stark's Dark Red Rome, and Rome Beauty No. 73 was lower than that of the fruits produced by crossing Gallia Beauty with McIntosh, Delicious, Jonathan, Starking, and Red Spy.

SELF- AND CROSS-POLLINATION OF OTHER APPLE VARIETIES

ARKANSAS

Auchter and Schrader (*1*) reported that Grimes was not effective in producing fruit when used as a pollinizer of the Arkansas variety, This is the only case yet reported in this country where a variety of apple with highly germinable pollen has failed to be an effective pollinizer of a variety to which it is not closely related.

The data presented in table 4 confirm the conclusions of Auchter and Schrader. Delicious and Jonathan were very effective pollinizers.

The flowers pollinated by Grimes Golden failed to enlarge even slightly and abscised during the early part of the first drop. These

flowers were fixed and examined cytologically. Although the examination has not yet been completed, none of the embryo sacs showed any evidence of fertilization. When fertilization takes place in 1 or 2 ovules of an apple flower, its receptacle enlarges slightly before the flower abscises.

TABLE 4.—*Results of self- and cross-pollination of various apple varieties, 1931*

ARKANSAS, TREE 262

Pollen parent	Treatment of flowers in cluster	Number of flowers	Percentage of flowers setting fruit—		
			After first drop	After second drop	After thinning
Delicious	2 not emasculated	319	38.9	10.6	
Grimes	do	290	1.8	0	
Jonathan	do	154	0	0	
Open pollinated	do	250	39.1	10.9	
		2,105	6.3	5.8	* 5.8

BALDWIN, TREE 395

Baldwin	None emasculated	510	7.7	3.1	
	do	14	3.5		
Open pollinated		1,071		10.7	6.5

DELICIOUS, TREE 279

Delicious	None emasculated	279	0	0	
Starking	2 not emasculated	202	0	0	
Cortland	do	213	62.4	29.6	
Gallia Beauty	do	203	78.3	39.4	
Golden Delicious	do	191	28.2	14.3	
Jonathan	do	191	16.2	13.6	
	do	109	41.3	22.9	
Turley	do	138	0	0	
Open pollinated		1,171		12.4	.6

JONATHAN, TREE 445

Jonathan	None emasculated	450	3.1	1.1	
	do	855		2.8	
Minkler	2 emasculated	249	.8	0	
Starking	do	754	33.1	16.9	
	do	200	19.5	14.0	
Turley	do	200	0	0	
Open pollinated		557	39.5	4.5	* 4.5
		606	54.9	11.2	5.8

TURLEY, TREE 477

Turley	None emasculated	263	1.1	.8	
Delicious	2 not emasculated	216	8.8	8.8	
Jonathan	do	103	23.3	13.6	
Open pollinated		1,002	13.7	11.3	

* Not full crop.

BALDWIN

The primary purpose of the Baldwin selfing experiments during the period 1923 to 1931 was to determine possible seasonal variation in the degree of self-fruitfulness. This variety has occasionally been considered one in which such variation might be sufficiently great to give full crops in some years.

The data for the year 1931, presented in table 4, show that on two selfed branches 3.1 and 3.5 percent of the flowers set fruit, while on the open-pollinated branch 6.5 percent of the flowers did so. The degree of self-fruitfulness of this variety was thus approximately 50 percent of a full crop. On the basis of the proportion of flowering to fruiting points (table 5), 27 and 36 percent of a full crop was obtained from these branches. This difference in percentage is due to the fact that a few of the selfed clusters had two fruits. These would obviously be included in the percentage of flowers setting fruit, but not in the data based on the relationship of flowering to fruiting points.

TABLE 5.—*Relation between flowering and fruiting points of self- and open-pollinated branches of different apple varieties, 1930–32*

Pollen parent	Total number of flowering points	Percentage of flowering points with fruits—	
		After second drop	After thinning
ROME BEAUTY, TREE 430, 1930			
Rome Beauty	74	4.1	4.1
Open	139	48.2	48.2
ROME BEAUTY, TREE 430, 1931			
Rome Beauty	61	4.9	4.9
	24	4.2	4.2
Open	252	72.3	72.3
ROME BEAUTY, TREE 326, 1932			
Rome Beauty	25	8.0	8.0
Open	141	71.6	65.2
GALLIA BEAUTY, TREE 16, 1930			
Gallia Beauty	50	20.0	20.0
	56	8.9	8.9
	40	15.0	15.0
Open	92	57.6	57.6
GALLIA BEAUTY, TREE 16, 1932			
Gallia Beauty	89	6.7	6.7
	93	16.1	16.1
Open	206	53.9	52.4
BALDWIN, TREE 395, 1931			
Baldwin	110	13.6	13.6
	50	10.0	10.0
Open	185	44.9	37.8
JONATHAN, TREE 445, 1931			
Jonathan	84	4.7	4.7
	156	15.4	15.4
Open	110	51.8	31.8

Delicious

The work with Delicious was designed to test the effectiveness of certain varieties as pollinizers of Delicious, the cheesecloth-bag method being used.

The selfed branch of Delicious (table 4) produced no fruits. In the cross-pollination tests Starking proved useless as a pollinizer. Overholser and Overley (*19*) very recently reported a similar result and also showed the reciprocal cross to be unfruitful. Turley was of no value, since its pollen had a very low germinability, and the tubes produced were almost functionless. On the other hand, Cortland Gallia Beauty, Golden Delicious, and Jonathan were effective pollinizers.

The data for seed content (table 3) showed no significant differences which would indicate that the good pollinizing varieties are unequal in their value as pollinizers.

Jonathan

The pollination experiment with Jonathan was designed (1) to test the effectiveness of Minkler, Turley, and Starking as pollinizers, and (2) to ascertain the degree of self-fruitfulness of the variety in the same year, as compared with Rome Beauty and Gallia Beauty, the cheesecloth-bag method being used.

The data (table 4) show that 1.1 and 2.8 percent were obtained from two selfed limbs. A full crop was equivalent to 5.8 percent. On this basis the self-fruitfulness was approximately 50 percent of a full crop. Data published in 1930 (*12*) showed that self-fruitfulness was 33 percent of a full crop on the tree used.

Minkler and Turley were of no value as pollinizers. Minkler, like Turley, had pollen of low germinability and produced short, thick tubes. Starking was very effective in producing fruit.

McIntosh

McIntosh, selfed, gave a small percentage of set, which was clearly only a fraction of a full crop.

Turley

The experiment with Turley showed it to be similar to Stayman in possessing a low degree of self-fruitfulness (table 4). Delicious and Jonathan were effective pollinizers.

DISCUSSION OF RESULTS

SELF-FRUITFULNESS OF ROME BEAUTY AND GALLIA BEAUTY

The experiments reported in the present paper conclude 8 years of work with Rome Beauty and 4 with Gallia Beauty. As reported in previous publications and as shown in considerable more detail in the present paper, both Rome Beauty and Gallia Beauty produced an appreciable number of fruits from selfed flowers, but in no case was the percentage of fruit equivalent to a full crop. On the basis of comparison with open-pollinated limbs, the degree of self-fruitfulness approximated 25 to 50 percent of a full crop. These varieties can be termed "partially self-compatible."

THE GALLIA BEAUTY-ROME BEAUTY RELATIONSHIP

The results presented herein conclude 6 years of work using Gallia Beauty as a pollinizer of Rome Beauty and 4 years using Rome Beauty as a pollinizer of Gallia Beauty. Rome Beauty and Gallia Beauty produced approximately the same percentage of fruits when used as pollinizers of each other as did either variety when selfed. Rome Beauty No. 73 and Stark's Dark Red Rome were also partially effective in producing fruit upon Gallia Beauty and Rome Beauty.

It might possibly be expected by some that if Gallia Beauty is a seedling of Rome Beauty, as has been reported by Gourley and Ellenwood (*7*), that the two varieties should be interfruitful. Rome Beauty No. 73, also reported to be a seedling, was only partially effective as a pollinizer. Apparently seedlings of Rome Beauty, which differ from the parent variety practically only in color of fruit, are not completely compatible. The Gallia Beauty-Rome Beauty relationship and the reciprocal cross are apparently an example of incompatibility similar to that reported by Crane and Lawrence (*3*, *4*) with closely related varieties of sweet cherry (*Prunus avium*). In this case the incompatibility is only partial.

THE ARKANSAS-GRIMES GOLDEN RELATIONSHIP

On the basis of present evidence, the failure of the Arkansas-Grimes golden cross does not appear to be an example of incompatibility of the same type as the Gallia Beauty-Rome Beauty relationship. In the first place, there is no genetical or systematic evidence of a relationship between the two varieties. Furthermore, Nebel (*18*) has reported that the Arkansas is triploid having 51 chromosomes, and that Grimes Golden is apparently diploid. The writer has observed from a cytological examination of the megasporogenesis that chromosomal aberrations are abundant in the Arkansas variety. Possibly, this is a factor in the failure of syngamy and seed development in this cross.

THE DEGREE OF SELF-FRUITFULNESS OF BALDWIN

The results obtained in the study reported in this confirm those already published (*9*, *10*, *12*) by the writer in regard to the degree of self-fruitfulness of the Baldwin. In no case during the 9 years' work (1922 to 1931, inclusive) with the enclosed-tree, glassine-bag, or cheesecloth-bag method has the degree of self-fruitfulness been equivalent to a full crop.

The Baldwin has been reported by a number of investigators, of whom Nebel (*18*) is one, as triploid. Its pollen is of very low germinability, and a large proportion of the tubes produced are short and thick. There is no doubt but that chromosomal aberrations are abundant during megasporogenesis. The poor germination of the seeds and the weak growth of the seedlings are indirect evidence of that fact. In view of its triploid nature, the high degree of self-fruitfulness of the variety is surprising. In fact, its self-fruitfulness is higher than that of any triploid variety yet reported in this country. It is apparent that the tendency of the Baldwin to develop fruits, even though the seed number per fruit may be very low, is a factor of importance in determining the degree of self-fruitfulness of the variety.

SELF- AND CROSS-POLLINATION OF DELICIOUS

Until recently the results of cross-pollination experiments with Delicious as the female parent have been unsatisfactory. Ballard (*2*), Dorsey (*5*), Haber (*8*), and Murneek, Yocum, and McCubbin (*17*) obtained very low sets with a number of varieties now known to be good pollinizers. Whitehouse and Auchter (*20*) were the first investigators to report good percentages of set. Since that time Luce and Morris (*15*), Marshall, Johnston, and others (*16*), Einset (*6*), Overholser and Overley (*19*), and the writer have obtained very satisfactory sets. The writer agrees with Einset, who reviewed the data on this point, that the results in these crosses support the conclusion that emasculation is very detrimental to the set of Delicious. In no case has the writer been able to get a satisfactory percentage of emasculated flowers to set fruit. Fortunately, emasculation is unnecessary in cross-pollination tests in this variety.

The writer (*11*) showed that competition between the flowers of a cluster is another factor of importance in determining the percentage of flowers setting fruit. It was shown that as the number of pollinated flowers of a cluster increased, the percentage which could set fruit very rapidly decreased.

The evidence presented very recently by Overholser and Overley (*19*), as well as that given in this paper, indicates that the Delicious and its principal "bud sports" are not interfruitful. In fact, the incompatibility is almost complete. This might be expected in view of the almost complete self-incompatibility of Delicious. This failure of Delicious and its closely related varieties is an example of incompatibility similar to those reported by Crane and Lawrence (*3*) for the sweet cherry.

SEASONAL VARIATION IN DEGREE OF SELF-FRUITFULNESS

When it is stated that apple varieties differ from year to year in their degree of self-fruitfulness, it is tacitly assumed that possibly certain varieties which are not sufficiently self-fruitful for a full crop one year may in another year produce a full crop. The suggestion has been offered that temperature changes, by affecting pollen-tube growth, are of great importance in producing this variation. The writer does not disagree with the importance of temperature in regulating pollen-tube growth. Unquestionably, the degree of self-fruitfulness of a variety differs from year to year and from orchard to orchard. The effect of partial defloration on increasing the percentage of fruits developing from selfed flowers indicates that the degree of self-fruitfulness may also vary from branch to branch and from flower to flower. However, during the 10 years of continuous experimental work at the Ohio station, the degree of self-fruitfulness has never varied to the extent that full crops have been produced on any variety used in the experiments. It might possibly be expected that full crops could be produced occasionally on Jonathan, Baldwin, Rome Beauty, and Gallia Beauty, but it has not been evident in these experiments or in any others.

The greater percentages of set obtained from flowers in a partially deflorated cluster might be expected in view of the more favorable nutritional conditions. For determining the degree of self-fruitfulness of a variety, a method which allows the flowers to develop under the most favorable nutritional conditions would, in general, seem the best

one. It must be admitted, however, that in pollinating only two flowers per cluster, commercial orchard conditions are merely approximated. Only when early frosts have eliminated several flowers of a cluster are the conditions in commercial orchards identical, as far as nutrition is concerned, with the experimental defloration procedure. Since, even under the most favorable nutritional conditions, a full crop of fruit is not produced from selfed flowers, the writer concludes that no variety in Ohio possesses a sufficiently high degree of self-fruitfulness to warrant its being recommended for planting in solid blocks.

CORRELATION BETWEEN POLLEN GERMINATION AND THE VALUE OF VARIETIES AS POLLINIZERS

Throughout the pollination work covering the 9-year period, there has been no case in which a sample of pollen which germinated very poorly gave a satisfactory set of fruit when taken to the orchard and applied by hand. This has been true whether the pollen has been from a variety such as Stayman Winesap, which normally has poor pollen, or from a variety which usually has good pollen. This indicates that the media used in the pollen germination tests are satisfactory, and this also makes it possible, in many instances, to predict the value of a variety as a pollinizer before one has cytologically examined microsporogenesis.

It has now become known that most of the varieties of apples which regularly have pollen of very low germinability are triploid. Nebel (*18*) has reported in this group Baldwin, Arkansas, Rhode Island Greening, Gravenstein, and Tompkins King. The writer has determined that Stayman Winesap is triploid and that Turley is apparently so. Minkler, which failed to be of value as a pollinizer in these experiments, is also likely triploid. As soon as the chromosome behavior of the various varieties during microsporegenesis has been examined, their effectiveness as pollinizers can be predicted. There are still several varieties of some commercial importance which have pollen of very low germinability and which have not yet been examined during the meiosis of the pollen mother cells.

SUMMARY

The experiments reported in this paper are largely concerned with the self-pollination and cross-pollination of Rome Beauty and Gallia Beauty. Of secondary importance are some data from several other varieties, including Arkansas, Baldwin, Delicious, Jonathan, and Turley.

Rome Beauty and Gallia Beauty produced an appreciable number of fruits when selfed, but under the most favorable nutritional conditions only 25 to 50 percent of a full crop was produced.

Gallia Beauty and Rome Beauty were only partly effective as pollinizers of each other. Approximately the same percentages were obtained as when each variety was selfed.

Rome Beauty No. 73, supposedly a seedling of Rome Beauty, was also only partially compatible on Rome Beauty and Gallia Beauty. Stark's Dark Red Rome also was only partially effective as a pollinizer of Gallia Beauty.

McIntosh, Jonathan, Golden Delicious, Starking, and Red Spy were effective pollinizers of Rome Beauty and Gallia Beauty. Melba was effective as a pollinizer of Rome Beauty. No evidence was obtained indicating a difference in the effectiveness of these varieties as pollinizers of Rome Beauty and Gallia Beauty.

The failure of Grimes Golden to produce fruit on the Arkansas was confirmed. This it is to be noted is a triploid × diploid cross.

Baldwin produced an appreciable set of fruit when selfed. Under favorable nutritional conditions self-fruitfulness was 25 to 50 percent of a full crop.

Delicious was self-unfruitful, and Starking failed to produce fruit when used as a pollinizer of this variety. The combination Delicious × Starking and its reciprocal, together with the Gallia Beauty-Rome Beauty relationship and its reciprocal, are apparently examples of an incompatibility similar to that found in other fruits, notably the sweet cherry.

Jonathan, Gallia Beauty, Cortland, and Golden Delicious were effective pollinizers of Delicious. No evidence was obtained indicating a difference in their value as pollinizers.

McIntosh had a low degree of self-fruitfulness.

Jonathan flowers showed a degree of self-fruitfulness equivalent to 25 to 50 percent of a full crop under the most favorable nutritional conditions. Because of their low pollen germinability, Minkler and Turley were ineffective pollinizers of Jonathan or any other variety on which they were used. Cytological examination of Turley during meiosis indicated that it is likely triploid.

Turley, a variety of the Stayman Winesap type, had a corresponding low degree of self-fruitfulness. Delicious and Jonathan were effective pollinizers.

The varieties which consistently showed the highest degrees of self-fruitfulness during the course of the writer's experimental work over a period of 9 years are Baldwin, Jonathan, Rome Beauty, and Gallia Beauty. This self-fruitfulness, however, even under the most favorable nutritional conditions, has never been equivalent to a full crop.

LITERATURE CITED

(1) Auchter, E. C., and Schrader, A. L.
1926. cross fertilization of the arkansas (mammoth black twig) apple. Amer. Soc. Hort. Sci. Proc. (1925) 22: 96–105.

(2) Ballard, W. R.
1916. methods and problems in pear and apple breeding. Md. Agr. Expt. Sta. Bul. 196, p. [79]–92, illus.

(3) Crane, M. B., and Lawrence, W. J. C.
1929. genetical and cytological aspects of incompatibility and sterility in cultivated fruits. Jour. Pomol. and Hort. Sci. 7: 276–301, illus.

(4) ——— and Lawrence, W. J. C.
1931. sterility and incompatibility in diploid and polyploid fruits. Jour. Genetics 24: [97]–107.

(5) Dorsey, M. J.
1922. the set of fruit in apple crosses. Amer. Soc. Hort. Sci. Proc. (1921) 18: 82–94.

(6) Einset, O.
1930. cross-unfruitfulness in the apple. N.Y. State Agr. Expt. Sta. Tech. Bul. 159, 24 p.

(7) Gourley, J. H., and Ellenwood, C. W.
1926. fruit varieties in ohio. i. Ohio Agr. Expt. Sta. Bul. 391, p. 427–438, illus.

(8) HABER, E. S.
1923. POLLINATION STUDIES WITH JONATHAN AND DELICIOUS APPLES. Iowa State Hort. Soc. Trans. 58: 154–156.
(9) HOWLETT, F. S.
1927. APPLE POLLINATION STUDIES IN OHIO. Ohio Agr. Expt. Sta. Bul. 404, 84 p. illus.
(10) ———
1928. FURTHER SELF- AND CROSS-POLLINATION EXPERIMENTS WITH THE BALDWIN APPLE. Amer. Soc. Hort. Sci. Proc. (1927) 24: 105–110.
(11) ———
1929. FRUIT SETTING IN THE DELICIOUS APPLE. Amer. Soc. Hort. Sci. Proc. (1928) 25: 143–148, illus.
(12) ———
1930. FURTHER EXPERIMENTS ON THE RELATIVE SELF-FRUITFULNESS OF APPLE VARIETIES. Amer. Soc. Hort. Sci. Proc. (1929) 26: 49–55.
(13) KNOWLTON, H. E.
1930. SOME RECENT RESULTS IN STERILITY STUDIES. Amer. Soc. Hort. Sci. Proc. (1929) 26: 62–64.
(14) ———
1932. POLLINATION STUDIES WITH SOME NEWER APPLE VARIETIES. Amer. Soc. Hort. Sci. Proc. (1931) 28: 71–73.
(15) LUCE, W. A., and MORRIS, O. M.
1928. POLLINATION OF DECIDUOUS FRUITS. Wash. Agr. Expt. Sta. Bul. 223, 22 p., illus.
(16) MARSHALL, R. E., JOHNSTON, S., HOOTMAN, H. D., and WELLS, H. M.
1929. POLLINATION OF ORCHARD FRUITS IN MICHIGAN. Mich. Agr. Expt. Sta. Spec. Bul. 188, 38 p., illus.
(17) MURNEEK, A. E., YOCUM, W. W., and MCCUBBIN, E. N.
1930. APPLE POLLINATION INVESTIGATIONS. Missouri Agr. Expt. Sta. Research Bul. 138, 36 p., illus.
(18) NEBEL, B. R.
1931. RECENT FINDINGS IN THE CYTOLOGY OF FRUITS (CYTOLOGY OF PYRUS III). Amer. Soc. Hort. Sci. Proc. (1930) 27: 406–410, illus.
(19) OVERHOLSER, E. L., and OVERLEY, F. L.
1932. POLLINATION OF CERTAIN BUD SPORTS IN NORTH CENTRAL WASHINGTON. Amer. Soc. Hort. Sci. Proc. (1931) 28: 74–77.
(20) WHITEHOUSE, W. E., and AUCHTER, E. C.
1927. CROSS POLLINATION STUDIES WITH THE DELICIOUS APPLE. Amer. Soc. Hort. Sci. Proc. (1926) 23: 157–161.

LOSSES OF VITAMIN A IN DRYING FRESH RAW CARROTS AND SWEETPOTATOES AND CANNED SPINACH [1]

By G. S. FRAPS, *chief, Division of Chemistry,* and RAY TREICHLER, *assistant chemist, Texas Agricultural Experiment Station*

INTRODUCTION

The method of estimating vitamin A requires that it be fed to rats over a period of 8 weeks. Storage for much longer periods is sometimes necessary until the animals are available for the purpose. Some material may deteriorate during the period of storage. In any investigation of the vitamin A activity of fresh materials, it is important to know how to store them without appreciable loss of this vitamin.

Some workers have dried the fresh materials and used the quantity of vitamin A in the dried materials to estimate the quantity in the fresh material, or the effect of conditions on it, but very little evidence is available as to the effect of drying on the quantity of vitamin A originally present. A number of investigators have studied the effect of different methods of drying on the vitamin A in the product obtained by the various processes, but these investigations throw no light on the amount of vitamin A lost during the drying. Some comparison of fresh material with the same food canned by different processes have been made, but the fresh material was usually purchased as needed and was not a portion of the food which was actually canned.

Jones, Murphy, and Nelson [2] compared the vitamin A content of raw frozen oysters with that of the dehydrated product and found that vitamin A was largely destroyed by dehydration. Dutcher, Honeywell, and Dahle [3] compared fresh milk brought daily to the laboratory with the same milk pasteurized and evaporated and reported a loss of about 10 to 30 percent of vitamin A. Morgan and her co-workers [4] compared the vitamin A content of fresh fruits frozen and kept at a temperature of $-17°$ C. witb the dried or dehydrated fruit prepared by different methods. Some of the fruits were sulphured before drying and some were not. Peaches lost about 14 percent of their original vitamin A, apricots 49 to 84 percent, prunes 9 to 76 percent, figs 50 to 80 percent, and apricots 50 to 70 percent.

The present paper reports an experiment to determine the loss of vitamin A in drying carrots, spinach, and sweetpotatoes, as compared with the vitamin A content of raw carrots, canned spinach, and raw sweetpotatoes, respectively.

[1] Received for publication June 5, 1933; issued October; 1933. Technical series no. 251, Texas Agricultural Experiment Station.

[2] JONES, D. B., MURPHY, J. C., and NELSON, E. M. BIOLOGICAL VALUES OF CERTAIN TYPES OF SEA FOODS. II—VITAMINS IN OYSTERS (OSTREA VIRGINICA). Indus. and Engin. Chem. 20: 205–210, illus. 1928.

[3] DUTCHER, R. A., HONEYWELL, H. E., and DAHLE, C. D. VITAMIN STUDIES. XVI. VITAMIN A IN EVAPORATED MILKS MADE BY VACUUM AND AERATION METHODS. Jour. Biol. Chem. 75: 85–94. 1927.

[4] MORGAN, A. F., and FIELD, A. VITAMINS IN DRIED FRUITS. II. THE EFFECT OF DRYING AND OF SULFUR DIOXIDE UPON THE VITAMIN A CONTENT OF FRUITS. Jour. Biol. Chem. 88: 9–25. 1930.

——— FIELD, A., and NICHOLS, P. F. RECENT STUDIES OF THE VITAMIN A AND C CONTENT OF DRIED APRICOTS AND FIGS. Fruit Prod. Jour. and Amer. Vinegar Indus. 11: 304. 1932.

——— and MADSEN, E. O. A COMPARISON OF APPRICOTS AND THEIR CAROTENE EQUIVALENT AS SOURCES OF VITAMIN A. Jour. Nutrition 6: 83-93. 1933.

MATERIALS USED

A case of canned spinach of apparently uniform quality was obtained. Some of the cans were opened, the juice was drained off, and the spinach was dried in a vacuum at 100° C. for 12 hours. During the process of the feeding experiment, a can of the spinach was opened each week, the juice was drained off, and the moist material was fed to rats. The dry material was determined, and the moist sample was kept in the refrigerator except while being fed.

Raw carrots were purchased on the market, and a portion was kept in the refrigerator. The supply was not sufficient, and another lot was bought toward the end of the experiment. Part of the carrots first purchased were ground and dried immediately in a vacuum for 12 hours at 73° C.

Puerto Rico sweetpotatoes were purchased from a farmer. A portion after being ground was dried for 7 hours at 100° C. The rest were stored at room temperature. It is possible that there was some loss of vitamin A during storage at room temperature.

All dried samples were stored in dark-glass containers, and only enough to last 2 or 3 weeks was removed and ground for feeding the experimental animals.

RESULTS

The units of vitamin A were estimated by the Sherman-Munsell unit method as used in this laboratory and previously described.[5]

Sixteen groups of rats, 6 in a group, except one group that had 8, were used in carrying out the experimental work. The average gains in weight, together with the amount of the particular vegetable allotted per day to each group, are found with other details in table 1. The material being tested was usually fed twice a week.

The raw yellow carrots contained approximately 43 units of vitamin A per gram of raw fresh material containing 11.4 percent dry matter. Reduced to a dry basis they had approximately 377 units per gram. The vacuum-dried sample of carrots contained approximately 77 units per gram. Approximately 80 percent of the vitamin A in the carrots was lost during the drying process (table 1).

The raw yellow sweetpotatoes with 28.7 percent of dry matter contained approximately 20 units of vitamin A per gram. This, when calculated on a dry basis, is about 70 units per gram. The vacuum-dried sample of the sweetpotatoes had 50 units of vitamin A per gram. The destruction of vitamin A appears to be approximately 29 percent. Other samples of yellow sweetpotatoes examined for their vitamin A content contained 50 units per gram on the original moist basis.

The canned spinach contained 140 units of vitamin A per gram of fresh pressed spinach solids, containing, on an average, 14.7 percent of dry matter. Calculated to a dry basis, the canned spinach had 952 units of vitamin A per gram. The vacuum-dried spinach contained 333 units of vitamin A per gram. Thus the spinach lost 65 percent of its vitamin A content during the drying process.

[5] FRAPS, G. S. VARIATIONS IN VITAMIN A AND CHEMICAL COMPOSITION OF CORN. Tex. Agr. Expt. Sta. Bul. 422, 46 p. 1931.

TABLE 1.—*Effect of drying in vacuo on vitamin A in carrots, sweetpotatoes, and spinach*

Laboratory no.	Material tested and treatment	Rate fed per day	Rats at start of test	Rats at end of test	Average gain or loss in weight in 8 weeks	Feed per unit of vitamin A	Vitamin A per gram feed	Loss of vitamin A
		Gram	*Number*	*Number*	*Grams*	*Gram*	*Units*	*Percent*
36027	Carrots, yellow, dried in vacuo	0.006	6	2	−1	0.013	77	80
		.03	6	4	62			
35974	Carrots, raw, yellow, original	.02	8	6	11	.023	[a] 43	
		.026	6	6	49			
37082	Sweetpotatoes, yellow, dried in vacuo	.01	6	1	5	.02	50	29
		.02	6	4	27			
		.03	6	6	44			
36942	Sweetpotatoes, raw, yellow	.02	6	0	0	.05	[b] 20	
		.04	6	3	8			
		.06	6	5	45			
37110	Spinach, canned, dried in vacuo	.0026	6	2	20	.003	333	65
		.003	6	4	30			
		.055	6	4	61			
37086	Spinach, canned (moist solids)	.008	6	3	57	.007	[c] 140	
		.01	6	4	39			
		.012	6	5	60			

[a] 377 units of vitamin A per gram of dry matter.
[b] 70 units of vitamin A per gram of dry matter.
[c] 952 units of vitamin A per gram of dry matter.

Carrots lost approximately 80 percent of their vitamin A during drying in a vacuum oven and sweetpotatoes about 29 percent. Although the dried materials were high in vitamin A content, they did not represent the original material, since there were serious losses in drying. These results indicate that green vegetables may be very high in vitamin A. The vitamin content of dried material may not be taken to represent the vitamin activity of the original moist material, as there may be serious losses in drying.

The results do not show whether or not there are losses in vitamin A in storing the fresh material, but they indicate that the stored fresh food, or the canned food, may be much higher in vitamin A than the dried material.

SUMMARY

Raw yellow carrots contained approximately 43 units per gram of fresh material containing 11.4 percent of dry matter, or 377 units per gram of dry matter. Vacuum-dried carrots contained 77 units of vitamin A per gram. The carrots lost approximately 80 percent of their vitamin A when dried. Canned spinach contained 140 units of vitamin A in the pressed solids which contained 14.7 percent of dry matter, or 952 units per gram of dry matter. Vacuum-dried spinach contained about 333 units vitamin A per gram. The spinach lost 65 percent of its vitamin A content in drying. Yellow Puerto Rico sweetpotatoes contained 20 units vitamin A per gram, with 28.7 percent of dry matter, or 70 units per gram of dry matter. The vacuum-dried sweetpotatoes contained 50 units per gram. The sweetpotatoes lost 29 percent of their vitamin A in drying.

The vitamin A content of dried vegetables may not represent the vitamin A content of the original moist material, as there may be serious losses in drying. Fresh green vegetables may contain much higher amounts of vitamin A than might be expected from tests of the dried material.

U. S. GOVERNMENT PRINTING OFFICE: 1933